OBSERVATIONS ON THE NUTRITION OF RHESUS MONKEYS RECEIVING HIGHLY PROCESSED RATIONS [1]

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ONE FIGURE

(Received for publication August 10, 1953)

INTRODUCTION

Although a significant part of the nutritional needs of soldiers subjected to combat must be supplied by highly concentrated rations, other rations may be used under less strenuous conditions which are considerably more varied and somewhat more easily acceptable. Such a ration, type 5-in-1 (Food and Container Institute, '52), has been devised during the last few years and has been employed in the Korean conflict in situations permitting the feeding of small groups in advance of field kitchens but not immediately committed to battle. This ration has been described previously (Tappan et al., '53).

When the 5-in-1 ration was first obtained in this laboratory for feeding experiments, it proved adequate for supporting satisfactory rates of growth in young rats (Tappan et al., '53). In order to obtain additional information about the

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned number 442 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of Defense.

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ration's adequacy, an extended experiment employing monkeys was initiated. Monkeys were of interest both because of their closer relationship to humans than that of laboratory rodents and because this species may be used for evaluating the ability of a ration to maintain an adult animal in a continuous state of health for periods of up to a few years.

METHODS

A total of 6 monkeys was employed in these experiments, with 4 animals maintained on the ration for periods ranging from 16 to 26 months. The monkeys were either obtained directly from an animal farm or had been maintained previously in this laboratory on a synthetic diet supplemented with adequate quantities of vitamins. Pre-experimental histories of adequate rates of weight gain were adjudged indicative of a satisfactory state of health. Previous treatment of individual animals will be considered where it is significant with respect to the experiment. Because of wide variation between animals in the time required for demonstrating nutritional needs, it has been necessary to let each monkey serve as its own control.

Methods of housing and care of the monkeys and vitamin levels employed as supplements have been described previously (Waisman et al., '43; Tappan et al., '52). Whenever a vitamin supplement was administered, the supplementation was continued until a change in the diet was made as indicated in figure 1. Routinely, about 5 times the usual vitamin levels were administered for a few (two to 5) days at the beginning of a supplementation period in order to hasten the replenishment of the animal's depleted supply. Work in this laboratory has indicated that often a monkey which becomes vitamin-deficient will respond to the missing vitamin or vitamins only when given an injection of an antibiotic, which presumably aids in controlling secondary infections that tend to become more serious as the animal's state of health declines.

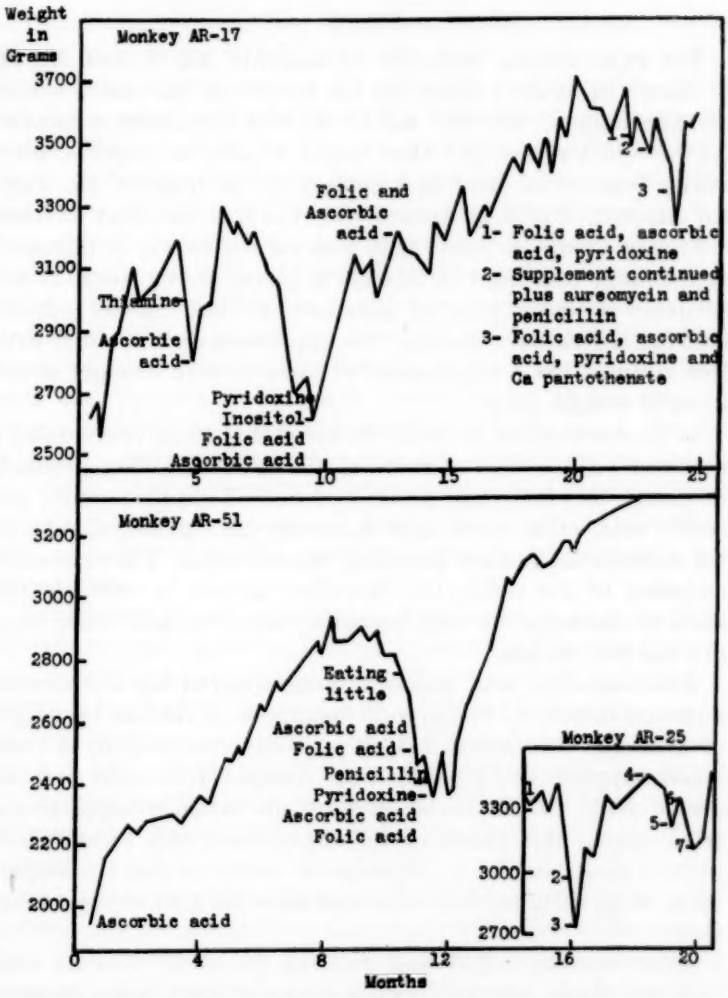

Fig. 1 Experimental histories of monkeys AR-17 and AR-51, with partial history of monkey AR-25. All vitamin supplements administered until new supplement indicated. Key to insert: 1. Ascorbic acid supplementation initiated one month earlier. 2. Thiamine, niacin, Ca pantothenate, *p*-aminobenzoic acid and ascorbic acid. 3. Supplement 2 continued plus aureomycin. 4. *p*-Aminobenzoic acid and ascorbic acid. 5. Folic acid, pyridoxine and ascorbic acid plus aureomycin. 6. Folic acid and ascorbic acid. 7. Pyridoxine, folic acid and ascorbic acid plus penicillin.

RESULTS

The experimental histories of animals AR-17 and AR-51 as shown in figure 1 illustrate the results of the entire series of experiments. Monkey AR-17 thrived for about 4 months on the 5-in-1 ration and then began rapidly to regress, soon losing interest in food and holding to the side of the cage for support. Swelling around the eyes was the most obvious deficiency symptom other than general weakness. A thiamine supplement was tried in this early phase of the work, in accordance with the general approach at that time of administering individual vitamins. No improvement was noted with this vitamin, but a supplement of ascorbic acid brought about a rapid weight gain.

In this connection it should be noted that these two monkeys received rations not containing the soluble coffee product. Although this beverage powder contained supplementary ascorbic acid, other work had indicated the extreme dislike of the animals for rations including this material. The necessary omission of the coffee has therefore served to evaluate the need of monkeys for supplementary ascorbic acid when they are fed this ration.

After ascorbic acid had been administered for one month, a second deficiency began to be manifest. A decline in weight of 4 months' duration was successfully reversed by a combined supplement of pyridoxine, inositol, folic acid and ascorbic acid. Of the series of multiple vitamin supplements tried during this phase of the experiment, this combination proved most beneficial. Subsequent work verified the importance of pyridoxine, folic acid and ascorbic acid supplementation.

After monkey AR-17 had received the above vitamin mixture for three months, results obtained with other animals indicated the possibility that a combination of ascorbic and folic acids might supply all or most of the supplementary needs of the animals. As is shown by the figure, the monkey increased in weight with only slight irregularities following this change in the vitamin mixture.

Blood analyses on this animal indicated a normal hemoglobin level when it had been one year on the experiment. During the administration of only folic and ascorbic acids, the hemoglobin value declined to a low of approximately 5.0 gm %. The erythrocyte and total leukocyte counts, as well as the differential leukocyte counts, remained normal throughout the experiment. Although the supplements were increased to include pyridoxine, Ca pantothenate, and finally thiamine and vitamin B_{12} (10 μg/day), the hemoglobin did not rise above 8.0 gm % during the remainder of the experiment. As is indicated in a report by Scott et al. ('51), these latter three vitamins were apparently most limiting next to folic acid and pyridoxine. Even 4 injections of 50 mg each of procaine penicillin G and aureomycin-HCl did not assist the animal in overcoming the anemia. In another animal, also maintained for an extended period with only a folic acid and ascorbic acid supplement, no improvement in the resulting anemia occurred when a complete vitamin mixture was administered.

Evidence obtained from three of the animals of the ability of the folic acid, ascorbic acid and pyridoxine combination to supply the major supplementary vitamin needs with the 5-in-1 ration is typified by the work with monkey AR-51. This young male animal had previously received a purified diet, supplemented with adequate vitamins, plus unrestricted quantities of bananas, apples, peanuts and other "treats." Such a regimen allowed the animal to acquire large tissue reserves of the vitamins.

As is demonstrated in figure 1, the monkey grew at a satisfactory rate for about 8 months while being given the ration. Following a two-month plateau in weight, a serious decline occurred. When almost 500 gm had been lost and interest in food had ceased, folic acid supplementation was initiated. Although the vitamin solutions were still consumed readily, no improvement in the animal's condition resulted. Five doses of procaine penicillin G totaling 440,000 units were also administered during a two-week period without apparent

benefit. In a final attempt to save the monkey's life without giving a complete vitamin mixture, pyridoxine was added to the supplement. It may be seen that the combination of pyridoxine, folic acid and ascorbic acid produce an almost immediate growth response. After two weeks of weight fluctuation while the tissue reserves of the missing nutrients were being replenished, a regular and quite rapid return to normality occurred. The monkey continued to increase in weight during the remainder of the experiment while receiving this supplement.

Blood data obtained throughout the study have shown a normal level of hemoglobin (12.2 to 15.3) for this animal even during the time of severe deficiency. A differential white cell count made when folic acid supplementation was initiated indicated a slight reversal in the neutrophyl-lymphocyte ratio (57/49). This symptom has been shown previously to be characteristic of a deficiency of the monkey anti-anemia factor (Cooperman et al., '45) which is, at least in part, a manifestation of an inadequate supply of folic acid (Smith and Elvehjem, '51a, b). After the animal's recovery, the neutrophyl-lymphocyte ratio returned to normal. Total leukocyte and erythrocyte levels were not abnormal even while the animal's condition was weakest.

The insert in figure 1 has been included to illustrate the useful role that may be played by antibiotic administration. After an initial period of gain and decline and a second small gain following the administration of ascorbic acid, a combination of thiamine, niacin, Ca pantothenate, *p*-aminobenzoic acid and ascorbic acid was utilized as part of the series of mixed supplements described for the other animals. As is shown in the figure, no increase in weight occurred until after the administration of two subcutaneous doses, of 75 mg each, of aureomycin-HCl. The temporary period of growth which followed may have resulted, at least in part, from the meeting of the folic acid requirement, established later in this and other animals, by the *p*-aminobenzoic acid, especially in the presence of the other vitamins.

The decline in weight resulting from a reduction in the supplementary vitamins to *p*-aminobenzoic acid and ascorbic acid was successfully corrected by folic acid, pyridoxine and ascorbic acid after the injection of one 75-mg dose of aureomycin. Within a few days after the removal of pyridoxine from the supplement, loss of weight again occurred and was corrected by the re-addition of this vitamin plus two 75-mg injections of penicillin. An intraperitoneal injection of 50 mg of penicillin a few days before the re-administration of pyridoxine had produced no observable benefit.

DISCUSSION

The finding that a pyridoxine deficiency eventually results when monkeys are fed the 5-in-1 ration adds significance to the earlier observations (Sporn et al., '48a, b; Register et al., '50) that experimental animals require supplements of this vitamin when receiving other highly processed rations. All of the studies concerning the nutritional value of army rations which have been made to date in this laboratory have pointed toward the fundamental nature of a pyridoxine deficiency associated with diets composed entirely of items processed to withstand long periods of storage. It is of particular interest that this deficiency occurs in a ration apparently adequate in most other respects, and consisting of approximately 45 different food items. The ascorbic acid supplements employed here do not indicate a deficiency in the ration with respect to the needs of the soldier, since sufficient quantities of the crystalline vitamin are incorporated into the beverage powders to satisfy human requirements. Since monkeys are known to have a high folic acid requirement (Cooperman et al., '46; Smith and Elvehjem, '51a, b), the folic acid supplements also may not reflect inadequacy for human nutrition.

Studies employing a "balanced" ration of processed foods obtained from commercial sources would be of considerable significance in determining whether the high pyridoxine re-

quirements are peculiar to the particular ingredients assembled into combat rations or are more widely important.

The anemia resulting after one to two years on this regimen is probably related to the macrocytic anemia observed by Poppen et al. ('52) even in control animals after 40 to 140 weeks on a purified diet. The condition was not corrected by "low doses" of vitamin B_{12}, folic acid or iron.

Despite the failure to maintain completely normal blood values, great improvement in the nutritional adequacy of the 5-in-1 ration as contrasted with older combat rations is indicated. The present imperfect state of knowledge as to the nutritional needs of monkeys is reflected in the failure to correct the blood picture by known nutrients. These investigations indicate the necessity for considering the adequacy of other members of the vitamin group in processed rations in addition to vitamin A, ascorbic acid, thiamine, riboflavin and niacin, around which previous concern has centered.

SUMMARY

The 5-in-1 type of combat ration, which consists of a generous variety of processed foods, has been shown capable of maintaining a state of health in monkeys for periods of up to two years with minor vitamin supplementation. Folic acid and pyridoxine are required by this species in addition to the crystalline vitamins added during the manufacture of the ration. The folic acid inadequacy is largely a reflection of the high requirement of monkeys. The general significance of the pyridoxine deficiency precipitated in animals by highly processed rations is discussed.

The injection of antibiotics to assist in controlling secondary complications during times of deficiency has proved to be a useful innovation in nutritional studies employing monkeys.

ACKNOWLEDGMENTS

We are indebted to Merck and Company, Inc., Rahway, New Jersey, for some of the crystalline vitamins and to the Lederle Laboratories Division, American Cyanamid Company, New York, N. Y., for aureomycin-HCl.

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STUDIES CONCERNING THE PYRIDOXINE REQUIREMENTS OF RATS RECEIVING HIGHLY PROCESSED RATIONS[1]

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(Received for publication August 10, 1953)

Previous reports have indicated that the responses of animals to highly processed rations may be considerably inferior to predicted results based on analyses of the constituents of such rations. Sporn and co-workers ('48a, b) observed that issues of army combat rations, types K, C and 10-in-1, which were available at that time, were incapable of supporting adequate growth rates or reproduction in rats, or a satisfactory state of health in monkeys. Rats receiving these regimens benefited from supplements of B vitamins and casein, while maintenance of life in monkeys frequently required such unrefined materials as whole milk.

Further work (Register and co-workers, '50a) indicated that a mixture of 6 amino acids and the vitamins pyridoxine and calcium pantothenate could replace the casein and vitamin mixture necessary for satisfactory nutriture of young rats receiving the K rations. Since these rations, although frequently lower in protein content than the level necessary to support optimum growth in rats (Sporn and Elvehjem, '48a),

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were designed to furnish at least 70 gm of protein per day to the soldiers (Quartermaster Corps studies, '44), the protein supplement added to make the ration more adequate for rats was not needed to correct a major inadequacy for human nutrition (National Research Council, '48).

The situation with respect to the vitamins, however, may have more serious implications. Since analysis for both pyridoxine and pantothenic acid indicated sufficient levels to supply the requirements of young rats, the marked increase in need, particularly for pyridoxine, indicated some yet undiscovered peculiarity in the metabolism of this vitamin under the conditions of these studies. Unpublished data of Register et al. ('50b) demonstrated that the mixture of the forms of vitamin B_6 found in the K ration was not significantly less active than pyridoxine, and could not be considered responsible for the increased need for the vitamin. Because of the recurring need for vitamin B_6 supplementation of these rations, and because this supplement was by far the most significant of any of the B vitamins in these experiments, our recent studies have been concentrated on the metabolism of this nutrient.

Previous attempts to find individual constituents of the K ration that might be responsible for the unusual vitamin B_6 requirement have been made by Register and co-workers ('50a), who demonstrated that despite the low level of protein in the ration, the growth response to supplementary quantities of the vitamin was increased by the presence of additional amounts of protein. A decreased response should have resulted if extra vitamin B_6 was needed because of protein inadequacy.

These workers (Register et al., '50b) also conducted studies concerning the influence of various carbohydrates on the vitamin B_6 requirement of young rats receiving several levels of fat in purified diets. Since dextrin, which more closely resembles the carbohydrate of the K ration than sucrose, decreased rather than increased the vitamin B_6 requirement, this approach, also, gave no indication of the reason for the

high vitamin requirement. It seemed logical, therefore, to begin a study of the individual items comprising the ration to determine whether one of these might be contributing heavily to the peculiar vitamin need.

For an understanding of experiments based on the army rations, a brief description of the rations employed is warranted. The K ration was designed [2] for emergency use, with each meal packed in a moisture-proof box of a size convenient for carrying in the soldier's pocket. Each of the three packages constituting a daily ration consists of a can of pork and egg mix, cheese or meat (corned pork, pork and beef loaf or chicken) plus crackers, beverage powder, sugar and confection or fruit bar. Although this assortment furnishes a somewhat monotonous diet, such a compact type of ration is extremely valuable in emergency situations and therefore deserves considerable study.

The 5-in-1 ration, useful under less strenuous conditions, consists of sufficient canned fruits, vegetables and soups, along with beverages, confections and variety items, packed in one box to give 5 soldiers a daily fare not unlike the processed foods purchased in any grocery store. Five different menus of this ration are available (Food and Container Institute, '52).

METHODS

Weanling male rats of the Sprague-Dawley strain, weighing between 40 and 50 gm, were employed, with each experimental group consisting of 6 animals. Methods of preparation and handling of the army ration were the same as those described earlier (Sporn and Elvehjem, '48a). Vitamins added to purified diets were employed at adequate but not excessive levels (Brown and Sturtevant, '47) in order to du-

[2] It should be emphasized that the K ration is now obsolete and has been superseded by other rations utilizing improved items. The K ration is used in studies of army rations because it embodies military characteristics common to all packaged operational rations. Chief among these characteristics are high caloric density, yielding maximum nourishment within minimum space and weight requirements, ease of use under combat conditions and high resistance to handling and storage damage.

plicate with a fair degree of accuracy the quantities present in the army rations. The following amounts, in milligrams per kilogram of ration, were employed as a single vitamin level: thiamine HCl 1.5, riboflavin 2.5, niacin 10, pyridoxine

TABLE 1

Influence of the K ration egg unit on the vitamin B_6 requirement of young rats

EXPT.	REGIMEN	AVE. TOTAL GROWTH	
		Wks. 1–4	Wks. 5–6
		gm	
	Whole K ration [1]	54 ± 3	
1	K ration + 2.5 mg B_6/kg	67	
	K without egg unit + beef	78	
	K without egg + beef + 2.5 mg B_6/kg	107	
2	Sucrose basal: 12.5% casein, 5% corn oil with 0.5 mg B_6/kg	82	
	As above with 1.0 mg B_6/kg	98	
	Same with 1.5 mg B_6/kg	124	
	Sucrose basal: 10% casein, 14% egg unit with 0.5 mg B_6/kg	100	
	As above with 1.0 mg B_6/kg	121	
	Same with 1.5 mg B_6/kg	131	
3	K with original pork and egg substituted for all protein units	57	40 [2]
	As above with new pork and egg	58	39
	K + 250 mg streptomycin·$CaCl_2$/kg	50	38
4	K with autoclaved fresh eggs	66	
	K with ham and eggs canned in 1950	54	
	K with freshly autoclaved pork and eggs	70	

[1] Average ± standard deviation of mean for 14 experiments.

[2] Five-tenths milligram of pyridoxine·HCl added per kilogram. Vitamin B_6 in all of these experiments is pyridoxine·HCl.

1.0, Ca pantothenate 10, folic acid 0.125, biotin 0.05, inositol 50, choline 500, *p*-aminobenzoic acid 62.5 and vitamin B_{12} 0.01.

The purified diets, usually patterned to simulate the K ration, contained 4% salts IV (Hegsted et al., '41), the vitamins listed above or a modification of this list, protein and fat as indicated and carbohydrate to make up to 100%. Vitamins

A and D were administered as two drops haliver oil [3] per rat per week with the purified regimens.

RESULTS

The results of a group of experiments dealing with the influence of the protein units of the ration on the vitamin B_6 requirement of young rats are shown in table 1. The ham and egg unit was chosen for individual study first because of the proposal of Cerecedo et al. ('48) that a high level of sulfur-containing amino acids results in an increased vitamin B_6 requirement.

From the first experiment it may be seen that a substitution of canned beef for the pork and egg mixture significantly increased the growth of the animals. The addition of vitamin B_6, however, even in the beef-containing ration, produced a marked improvement in the growth response of the rats. Since, as illustrated by experiment 2, no adverse relationship could be demonstrated between the pork and egg mixture and the vitamin B_6 requirement of the rats when this product was fed as the sole source of protein in an otherwise purified diet, the effect of replacing all of the protein portions (i.e., egg, cheese or meat units) of the ration by the pork and egg unit was determined. When this regimen (expt. 3) did not significantly alter the response to additional vitamin B_6 (14 gm/wk. gain without B_6 vs. 20 gm/wk. with B_6), it was concluded that the other protein units were producing approximately the same effect as the egg unit.

The results of experiment 3 also indicated that the processing of the egg mixture was more significant in the production of the effects being studied than storage, since the rats responded no better to the ration containing pork and egg canned approximately 6 months before the experiment than to a similar ration containing pork and egg canned in 1945, when this supply of ration was packed. Streptomycin in this situation did not decrease the apparent vitamin B_6 requirement (Linkswiler et al., '51). The detrimental influence of

[3] Abbott Laboratories, North Chicago, Illinois.

processing in contrast to storage was also observed in experiment 4, in which the various egg mixtures replaced only the egg unit of the K ration. Fresh eggs either with or without pork, substituted on an equal weight basis and therefore producing a ration containing more moisture and slightly less

TABLE 2

Relative value of various proteins of K ration

EXPT.		AVE. TOTAL GROWTH		
		Wks. 1–4	Wks. 5–6	Wks. 7–8
		gm	*gm*	*gm*
	Whole K ration [1]	54 ± 3		
5	Sucrose basal with 25% lard:			
	with 11.8% casein + 0.2% cystine	55		
	with 12% protein from K ration ham and eggs	66		
	with 12% protein from K ration cheese	71		
	with 12% protein from K ration pork	39		
6	K ration	72	56 [2]	
	Same + 0.75 mg B_6/kg	92		
	K with pork replacing all protein units	64	36 [2]	
	Same + 0.75 mg B_6/kg	80		
7	Sucrose basal with 25% lard and:			
	12% protein from K ration pork	52	23	30 [3]
	Same + 1.5 mg B_6/kg	46	20	31 [3]
	12% protein from fresh (processed) pork	96	52	38 [3]
	Same + 1.5 mg B_6/kg	97	46	47 [3]

[1] Average ± standard deviation of mean for 14 experiments.
[2] Two milligrams pyridoxine·HCl added per kilogram.
[3] Injected with 40 μg pyridoxine·HCl per day.

protein, supported better growth than either the new or original canned egg product.

In experiment 5 (table 2), in which sufficient dried and defatted (ether-extracted) material from the protein units was fed to make a 12% protein ration (N × 6.25) in each case, attention was called to the more serious growth-retarding influence of the pork unit of the K ration. It should be men-

tioned that unless otherwise stated pork loaf was the meat unit in the rations studied here. When the amount of pork in the ration was increased to replace all of the protein units, illustrated in experiment 6, the response to vitamin B_6 was somewhat less than with the unaltered K ration, despite the unusually high rate of growth shown by the animals receiving the unsupplemented ration in this experiment. As with the study of the egg unit, however, the need for additional vitamin B_6 could not be demonstrated when the pork unit was substituted in a semi-synthetic diet (expt. 7).

TABLE 3

Effect of fat level on growth of rats receiving K ration

RATION	AVE. TOTAL GROWTH	
	Wks. 1–3	Wks. 4–5 [1]
	gm	*gm*
Dehydrated K	39	43
Dehydrated, defatted K + 5% fat from K	54	53
Dehydrated, defatted K + 15% K fat	48	46
Dehydrated, defatted K + 25% K fat	32	31
Dehydrated, defatted K + 5% Crisco	54	57
Dehydrated, defatted K + 15% Crisco	43	51
Dehydrated, defatted K + 25% Crisco	28	30

[1] Four milligrams pyridoxine·HCl added per kilogram.

To gain information concerning the influence of the high level of fat in the ration on the pyridoxine metabolism of the animals, the experiment shown in table 3 was initiated. Here varying levels of either fat from the K ration or hydrogenated vegetable fat [4] were added to the ration after being defatted with a 3:1 ethanol-ether mixture, and pyridoxine was added after three weeks. It may be seen that although slightly more growth was obtained with the lower levels of fat, the response to vitamin B_6 was of similar magnitude in all cases. The growth of the vegetable fat-fed animals was also somewhat less than that of the animals receiving the fat from the K ration. It may be concluded, then, that although the

[4] Crisco, Procter and Gamble, Cincinnati, Ohio.

fat is partly responsible for the low growth rate supported by the K ration, it has little influence on the unusual vitamin B_6 needs of animals receiving this ration.

Although an explanation for the peculiar vitamin B_6 metabolism accompanying the K ration has been made more difficult by the problem of reproducing a similar vitamin B_6 need with any fraction of the ration apart from the other portions of this complex mixture, information has accumulated concerning the possibility of improving the ration by proper

TABLE 4

Effects of modifications of meat unit of K ration on growth of rats

REGIMEN	AVE. TOTAL GAIN 5 weeks
	gm
K ration	57
K ration + 2 mg pyridoxine/kg	101
K ration + 5% casein	76
K ration + 5% casein + 2 mg B_6/kg	128
K with Spam for pork unit	100
K with Spam for pork unit + B_6	114
K with Spam for pork unit + casein	129
K with Spam for pork unit + casein + B_6	168
K with equal mixture of chicken, pork and beef loaf	91
Above + B_6	134
Above + casein	113
Above + casein + B_6	158

substitution for the protein units. With this in mind, the experiment described in table 4 was initiated, in which another canned pork product and a combination of available K ration meat units replaced the pork unit. The results indicate a very marked improvement in both of the substituted rations, but in each case the vitamin B_6 response with and without added protein was still apparent.

In another study not described in detail because of the close resemblance of the results to data reported for weanling rats, it was determined that rats averaging 160 gm reach

their maximum weight much faster if given vitamin B_6 supplements, either with or without additional protein, when the K ration is fed, even after an adequate stock diet is employed for the initial growth period. The increased vitamin

TABLE 5

Response of young rats to 5-in-1 ration and various modifications

RATION	AVE. TOTAL GROWTH Weeks 1–5
	gm
Experiment 1	
5-in-1	127
5-in-1 + B vitamins [1]	131
5-in-1 + 4% casein	145
5-in-1 + 4% casein + B vitamins	157

	AVE. TOTAL GROWTH Wks. 1–4	SUPPLEMENT [2]	AVE. TOTAL GROWTH Wks. 5–6
Experiment 2			
5-in-1 with ordinary level of fat (24.2%)	106	B_6	56
		All vitamins	65
5-in-1 with lower [3] level of fat (18.8%)	117	B_6	62
		All vitamins	61

[1] Double level of vitamins.

[2] The two original groups of animals were divided into groups of 4 rats each for the last part of the experiment. These groups received 2 mg pyridoxine/kg or the complete vitamin mix at the double level.

[3] The fat level was lowered by removing all the obvious pieces of fat from the cans containing meat and by cooking the luncheon meat, bacon, weiners and sausages in a roaster until these meats were in approximately the same condition as would be those eaten by the soldiers.

B_6 need, then, is not a phenomenon associated exclusively with the post-weaning period of rapid growth.

An evaluation was also made of the possibility that the sodium nitrite used in the manufacture of the pork product might be at least partly responsible for the increased vitamin B_6 requirement associated with this unit. No reduction in the growth of the rats or alteration in vitamin B_6 requirement

resulted from the presence of sodium nitrite when K rations containing freshly processed pork in place of the pork unit were fed. Levels of nitrite up to 400 p.p.m. with respect to the pork were employed (twice the maximum limit of federal meat inspection regulations; Miller, '51). A pork luncheon meat,[5] which is also a product containing $NaNO_2$, significantly reduced the amount of vitamin B_6 required by the rats, in contrast to the K ration pork mixture.

Results of feeding experiments with the newer ration, type 5-in-1, are shown in table 5. It may be seen that this more elaborate ration supports good growth in young rats, and is improved only slightly by the addition of a mixture of the B vitamins or protein or by a lowering of the fat content. In order to gain information about the ability of this ration to support growth in animals for extended periods, experiments have been conducted employing monkeys and are reported separately.

DISCUSSION

The consistently large growth response observed in rats when vitamin B_6 supplements are added to the K ration, and the fairly high degree of standardization of this ration, have permitted its use as being representative of army rations requiring vitamin B_6 supplementation. Because of the low rate of growth with the unsupplemented ration, any alteration in the ration was considered to be an improvement if an increase in rate of gain occurred.

The information gained during these studies confirms earlier results (summarized by Register et al., '50a) showing an increase in the requirement for pyridoxine of animals receiving certain combat rations. As is discussed in the following paper, this phenomenon appears, tentatively, to be of general significance when diets composed of highly processed items are fed. With sufficient variety in the mixture of items comprising a processed ration, as is indicated by the studies employing the 5-in-1 ration, considerable improvement in nutri-

[5] Spam, Geo. A. Hormel and Co., Austin, Minnesota.

tional adequacy may be attained. A simple, more concentrated ration, exemplified by the K ration, also deserves careful investigation, however, for reasons of ease of manufacture, storage and shipment.

Although both the ham and egg and the pork loaf units of the K ration have been shown to be in part responsible for the increase in vitamin B_6 requirement, no such effect can be demonstrated when these units are fed apart from the remaining portions of the processed ration. It has been possible, however, to effect marked improvements in the K ration by substitutions of other meats or meat mixtures for the pork unit. With the possibility of a simple but effective improvement in this emergency ration, considerable impetus is given to research directed to further development of compact rations of this type if circumstances again warrant their use. Famine or other emergencies, including war, makes the study of rations that may be shipped and stored easily of great political, economic and nutritional significance.

SUMMARY

The high vitamin B_6 requirement of animals receiving highly processed rations has been studied by means of the combat ration, type K. In this regimen the ham and egg and the pork units seem to be associated with the unusual vitamin needs which the ration fosters. These protein constituents, however, do not increase the vitamin B_6 need of the animals in the absence of the other portions of the ration. Improvement in the ration by means of proper substitution, especially for the pork unit, has been demonstrated to be possible.

The unusual vitamin requirements which result from the use of processed rations and the nutritional significance of such rations are considered.

ACKNOWLEDGMENTS

We are indebted to Merck and Company, Inc., Rahway, New Jersey, for some of the crystalline vitamins and to the Abbott Laboratories, North Chicago, Illinois, for haliver oil.

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EFFECT OF FEEDING DDT-TREATED ALFALFA HAY TO SWINE AND OF FEEDING THE SWINE TISSUES TO RATS[1, 2]

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TWO FIGURES

(Received for publication December 26, 1951)

Lieberman ('45), Smith and Michelbacher ('46) and Lieberman and Hare ('46) have shown that alfalfa seed production can be increased if alfalfa weevil and lygus bugs are controlled by the application of DDT to alfalfa. This has led to the use of DDT on forage alfalfa for the production of hay. In view of this fact, it is important to know the effect of the DDT residue on livestock and upon their utilization of the feed.

There have been many investigations on the toxicity of DDT and on its accumulation in the tissues of laboratory and farm animals. These investigations have been reviewed by Leary et al. ('46) and Heyroth ('50). However, only a few experiments have been conducted to determine whether DDT

[1] Research supported in part by a research grant from the Division of Research Grants and Fellowships, National Institutes of Health, U. S. Public Health Service, and published with the approval of the Director of the Utah Agricultural Experiment Station.

[2] These data were presented at the American Chemical Society meetings in Boston, April 1–5, 1951.

[3] Chairman, Institute of Nutrition, and professor of animal husbandry; research chemist; research chemist; professor of biochemistry; professor of physiology; head, Veterinary Science Department; and associate professor, veterinary science, respectively.

is toxic to swine and whether the tissues of animals fed DDT are toxic to other animals or to humans.

Arant ('48) fed unhusked corn with a residue of 15 p.p.m. DDT to pigs for 102 days. At the end of the trial the fat contained 13 p.p.m., while steers fed the same corn had 46 to 65 p.p.m. of DDT in their fat after 105 days.

Two lots of 6 pigs each were fed for 36 days on a ration containing 75% corn meal and 25% ground beef that contained DDT (Carter, '48). The feed of the pigs in one lot contained 5 p.p.m. DDT and in the other 3 p.p.m., based on the weight of the total ration. The amounts of DDT from the two lots were 2.0 and 1.7 p.p.m. in the lean meat, 15.6 and 11.4 p.p.m. in the external and intramuscular fat, and 17.6 and 11.4 p.p.m. in the leaf fat. The DDT accounted for in the fat and meat portions of the animals was 49 to 57% of the total intake.

Swine fed primarily on corn containing 555 p.p.m. of DDT from the time they were weaned, January 2, to the time they were slaughtered, August 8, remained normal and healthy. Post-mortem studies and histological examination of sections of kidney and liver tissues showed no disorders. The leaf fat contained 271 to 387 p.p.m. DDT, liver tissue 1.6 to 3.6 p.p.m. and kidney tissue 1.2 to 32.0 p.p.m. (Decker, '46).

Hepatic cell alterations occurred in rats when they were fed diets containing as little as 5 p.p.m. DDT for a period of 20 weeks, and DDT accumulated in the body fat on diets containing as little as 1 p.p.m. (Laug et al., '50).

DDT accumulated in the fatty tissue and milk of dairy cows (Biddulph et al., '50), the fatty tissue and eggs of chickens (Bryson et al., '50) and the fat of lambs (Harris et al., '49). The DDT-dusted hay fed to the animals in these experiments was not toxic to the dairy cows, chickens or lambs. DDT, however, retards the synthesis of protein in the paunch of calves when fed at a dietary level of 25 p.p.m. (Bohman, '51).

The purpose of this experiment was to determine whether DDT-treated alfalfa hay was toxic to swine and whether the tissues of the swine were toxic to rats.

EXPERIMENTAL

Preparation of alfalfa hay

A 20-acre field of alfalfa was divided into 8 one-and-one-half acre plots with intervening buffer strips. The buffer strips were necessary in order to reduce the drift of DDT from one plot to another during its application to the alfalfa. Four of the plots were selected at random and 20 pounds of 10% DDT dust were applied per acre with a power duster on June 6, 1949.

The hay was cut on June 20, 1949, after which it was raked into windrows, sun-cured, baled in the field and stored on a cement floor in a barn. Before mixing the diets the hay was ground into a meal.

Feeding trial

Eleven male and 11 female Duroc-Jersey weanling pigs were fed during the fall of 1949, and the same number of pigs were fed during the spring of 1950. The pigs were segregated according to sex and allotted at random to individual pens 4 feet wide and 12 feet long. Each pen was equipped with a self-feeder and water trough.

The pigs were fed a basal diet of the following percentage composition from the time they were weaned until they weighed approximately 90 pounds; ground barley 72.46, soybean oil meal 14.0, dried whey 3.0, fish meal 2.5, tankage 7.5, salt 0.5 and cod liver oil concentrate (containing 3,000 I.U. of vitamin A and 400 — Association of Official Agricultural Chemists — chick units of vitamin D) 0.04. This diet contained 90.8% of dry matter and the percentage composition of the dry matter was: protein 22.5, ether extract 3.48, crude fiber 4.42, nitrogen-free extract 62.4, calcium 1.15 and phosphorus 0.89. The various levels of hay in the diets fed from weaning until the pigs were killed (table 1) were obtained by replacing barley with alfalfa hay from the DDT-treated plots and from the plots not treated with DDT (table 2). Between 90 and 150 pounds of body weight, the protein con-

tent of the diets of the pigs was reduced to 15.7% (dry basis). This was done by reducing the amount of soybean oil meal, dried whey, fish meal and tankage and replacing these ingredients with ground barley. Between 150 and 210 pounds

TABLE 1

Experimental design for swine feeding

ALFALFA HAY IN DIET	POUNDS DDT APPLIED PER ACRE			
	0		2	
	Fall	Spring	Fall	Spring
%	*sex*	*sex*	*sex*	*sex*
0	♀	♂		
3	♂	♀	♂	♀
6	♀	♂	♀	♂
9	♂	♀	♂	♀
12	♀	♂	♀	♂
15	♂	♀	♂	♀
18	♀	♂	♀	♂
21	♂	♀	♂	♀
24	♀	♂	♀	♂
27	♂	♀	♂	♀
30	♀	♂	♀	♂
33	♂	♀	♂	♀

TABLE 2

Composition of alfalfa hay

PLOT NO.	DDT APPLIED PER ACRE	DRY MATTER	DDT	PROTEIN	ETHER EXTRACT	CRUDE FIBER	N-FREE EXTRACT	CALCIUM	PHOSPHORUS
	lb.	%	*p.p.m.*	%	%	%	%	%	%
K35	0	93.1	0.0	13.5	1.20	37.2	38.9	1.54	0.25
K37	0	93.0	0.0	15.7	1.30	30.4	42.6	1.40	0.29
K39	0	93.1	0.0	13.6	1.76	33.4	40.6	1.57	0.19
K41	0	93.4	0.0	12.4	1.27	37.8	39.7	1.34	0.24
	Ave.	93.2	0.0	13.8	1.38	34.7	40.4	1.46	0.24
K34	2	93.1	23.6	14.6	1.26	37.2	38.0	1.43	0.25
K36	2	93.0	21.5	15.9	1.35	32.9	40.9	1.58	0.23
K38	2	93.2	23.6	13.4	1.44	36.7	40.3	1.93	0.21
K40	2	93.1	21.5	16.1	1.72	31.4	40.9	1.82	0.25
	Ave.	93.1	22.6	15.1	1.45	34.6	40.0	1.69	0.24

of body weight, the protein content of the diets was reduced in a similar manner to 14.7% (dry basis). In all there were 69 different diets. The feed intake was measured every two weeks.

The fall pigs developed an infestation of round worms soon after they were started on the experiment. Two pigs receiving the DDT-treated hay at the 30 and 33% levels died, and one pig receiving the control hay at the 30% level died. These deaths were caused by ascarids and necrotic enteritis. Three additional pigs were obtained to replace those that died. All the pigs were then treated with sodium fluoride to remove the ascarids. The spring pigs were treated for ascarids at weaning time and when they were about half way through the experiment.

Body weights were taken at two-week intervals throughout the experiment. The pigs were slaughtered when they reached 210 pounds in body weight. At the time of slaughter, samples of the liver and kidney were saved for histological study. The tissues were fixed in Zenkers solution and stained with hematoxylin and eosin. DDT analysis was made on the fresh kidney and leaf fat. The bacon and shoulder were cured by a commercial processor and the DDT content of the cured meat was determined. The methods used for the DDT analysis have been outlined by Harris et al. ('49).

Feeding tissues from swine that had consumed DDT-treated hay to rats

Kidney and leaf fat, cured bacon and cured shoulder were fed to weanling rats in an experiment designed to determine whether such tissues contained sufficient DDT to affect the rats. The tissues were obtained from the swine previously described in this report.

The diets listed in table 3 were fed for a period of 14 weeks, after which the animals were sacrificed. The diets contained approximately 22.0% fat because that is the approximate amount in an average human diet (Harris et al., '43) and a

dietary allowance of 18 to 30% protein is adequate for rats (Hamilton, '39). The rats were fed individually ad libitum and were weighed at two-week intervals. The feed intake was measured every two weeks.

The mesenteric and kidney fats of the rats were combined and analyzed for DDT. The liver was studied histologically.

TABLE 3

Composition of rat diets

INGREDIENT	SWINE FAT		SWINE BACON		SWINE SHOULDER	
	As fed	Dry	As fed	Dry	As fed	Dry
	%	%	%	%	%	%
Casein	22.0	22.2	14.25	14.2	10.87	12.0
Swine fat, raw	22.2	20.8	....	....	....	....
Swine bacon, cured	....	....	32.05	27.6	....	....
Swine shoulder, cured	....	....	....	....	34.47	24.2
Wheat germ oil	0.5	0.5	0.46	0.5	0.42	0.5
Salt mixture J33 [1]	4.0	4.2	3.65	4.0	3.40	4.0
NaCl	1.0	1.1	0.91	1.0	0.85	1.0
Whole liver substance	4.0	4.2	3.93	4.0	3.65	4.0
Brewers' yeast	4.0	4.1	3.93	4.0	3.65	4.0
Dextrinized starch	41.5	41.8	39.91	43.7	41.87	49.3
Cod liver oil	1.0	1.1	0.91	1.0	0.85	1.0
Total	100.0	100.0	100.0	100.0	100.0	100.0
Dry matter	93.0	100.0	90.6	100.0	85.6	100.0
Protein	22.0	23.7	23.6	26.0	18.1	21.1
Fat	23.6	25.4	19.7	21.7	19.8	23.1

[1] Percentage composition as follows: NaCl 35, KCl 10, K_2CO_3 25, Na_2CO_3 16.8198, $MgCO_3$ 9.2, $FeC_6H_5O_7 \cdot 3H_2O$ 3.6, $CuSO_4 \cdot 5H_2O$ 0.04, $MnSO_4$ 0.28, $K_2Al_2(SO_4)_4 \cdot 24H_2O$ 0.02, KI 0.01, $CoCl_2 \cdot 6H_2O$ 0.02, $ZnCO_3$ 0.01 and NaF 0.0002.

The DDT residue on the hay and in the tissues was determined by the methods described by Harris et al. ('49). All data were statistically analyzed and differences were considered significant at the 5% level of probability.

RESULTS

Effects of DDT on swine

There was some variation in the DDT content of the tissues, but in general the DDT in the fat, bacon and shoulder

of the swine increased with each level of hay fed (table 4). The fat contained the largest amount of DDT, while the shoulder contained the least. There were slight amounts of DDT in the fat, bacon and shoulder of the control pigs. The DDT in the tissues of the control pigs may have come from traces of DDT in the control hay or from the other ingredients in the diets or from slight contamination among the pigs,

TABLE 4

DDT content of swine tissues, and daily gain and amount of feed per pound of gain from weaning to approximately 210 pounds in weight[1]

ALFALFA HAY IN SWINE DIET	DDT						WEANING TO 210 LB. IN WT.		
	Applied per acre	On alfalfa hay (dry basis)	In swine diet (dry basis)	In swine fat	In swine bacon	In swine shoulder	Days on feed	Daily gain	Feed per pound gain (dry basis)
%	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*		*lb.*	*lb.*
0	–	–	0	0	0.4	0.6	125	1.40	4.06
3	0	0	0	0	0.3	0.2	114	1.58	3.26
6	0	0	0	0	0.2	0.4	128	1.04	3.58
9	0	0	0	0.6	0.5	0.2	145	1.38	3.48
12	0	0	0	0	1.1	0.7	120	1.44	3.78
15	0	0	0	0	0.7	0.4	124	1.48	3.80
18	0	0	0	0	0.6	0.4	132	1.37	3.92
21	0	0	0	0.6	0.6	0.6	138	1.43	3.86
24	0	0	0	0	0.5	0.5	150	1.22	4.52
27	0	0	0	0	1.4	0.8	136	1.26	4.47
30	0	0	0	0	0.4	0.4	156	1.12	4.87
33	0	0	0	0	0.5	0.4	164	1.18	4.87
Ave.	0	0	0	0.1	0.6	0.5	136	1.32	4.04
3	2	21.5	0.7	2.2	1.5	0.7	124	1.55	3.46
6	2	23.6	1.4	3.6	3.0	2.6	116	1.61	3.16
9	2	21.5	2.0	6.5	3.4	2.7	124	1.40	3.62
12	2	23.6	2.9	6.0	5.1	3.5	124	1.58	3.50
15	2	21.5	3.3	8.0	6.6	6.8	140	1.44	3.93
18	2	23.6	4.3	14.3	8.6	6.3	128	1.45	3.62
21	2	21.5	4.6	13.2	7.0	6.4	132	1.31	4.10
24	2	23.6	5.7	11.5	6.4	5.2	142	1.34	3.77
27	2	21.5	5.9	18.0	9.1	6.0	144	1.34	4.38
30	2	23.6	7.2	11.8	7.8	5.8	158	1.28	4.36
33	2	21.5	7.3	12.0	8.0	7.9	154	1.14	4.54
Ave.	2	22.5	4.1	9.7	6.0	4.9	135	1.40	3.86

[1] Each figure is the average of a male and female pig.

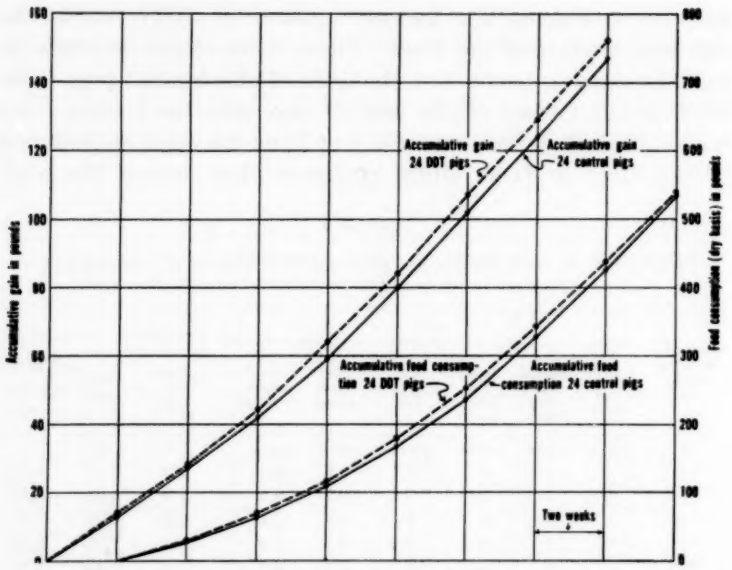
Accumulative gain in pounds
Food consumption (dry basis) in pounds
Accumulative gain 24 DDT pigs
Accumulative gain 24 control pigs
Accumulative food consumption 24 DDT pigs
Accumulative food consumption 24 control pigs
Two weeks

Fig. 1 Cumulative gain and cumulative food consumption of control and DDT-treated swine.

TABLE 5

Comparison of male and female pigs in their response to DDT when fed as a residue on alfalfa hay

CATEGORY OF INTEREST	MALES		FEMALES	
	Control	Receiving DDT	Control	Receiving DDT
DDT in diet (dry basis), p.p.m.	0.0	4.1	0.0	4.1
DDT in raw fat, p.p.m.	0.2	9.7	0.0	9.9
DDT in cured bacon, p.p.m.	0.7	6.6	0.5	5.9
DDT in cured shoulder, p.p.m.	0.4	5.2	0.5	5.1
Days on feed	139.0	140.0	136.0	130.0
Daily gain, lb.	1.37	1.33	1.39	1.44
Feed per pound gain (dry basis), lb.	4.02	4.12	3.91	3.73

because control pigs were randomized among pigs receiving some DDT in the diets.

There were no significant differences in the daily rate of gain, in utilization of feed (table 4) or in accumulative gain (fig. 1) between the control pigs and the pigs receiving DDT-treated alfalfa.

TABLE 6

DDT content of rat fat and cumulative gain, food consumption and body length of rats fed swine fat

ALFALFA HAY IN SWINE DIET	DDT					CUMULATIVE GAIN		FOOD CONSUMPTION		BODY LENGTH
	On alfalfa hay (dry basis)	In swine diet (dry basis)	In swine fat[1]	In rat diet (dry basis)	In rat fat	After 8 wks.	After 14 wks.	After 8 wks.	After 14 wks.	
%	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*gm*	*gm*	*gm*	*gm*	*cm*
0	–	0	0.0	0.2	12.5	223	275	691	1,427	22.5
3	0	0	0.0	0.3	7.4	263	345	776	1,457	24.0
6	0	0	0.0	0.4	13.7	205	270	632	1,140	23.0
9	0	0	1.1	0.5	15.4	230	305	728	1.422	23.0
12	0	0	0.0	0.2	20.3	231	306	681	1,234	24.0
15	0	0	0.0	0.1	12.4	209	276	738	1,323	24.0
18	0	0	0.0	0.3	15.8	225	280	693	1,274	22.5
21	0	0	1.3	0.6	13.7	236	318	820	1,431	24.0
24	0	0	0.0	0.4	25.3	286	367	636	1,496	25.0
Ave.	0	0	0.3	0.3	15.2	234	305	710	1,356	23.5
3	21.5	0.7	2.4	0.6	23.5	260	350	777	1,421	25.0
6	23.6	1.4	4.3	1.0	18.7	233	305	780	1,369	24.0
9	21.5	2.0	6.1	1.4	9.1	217	295	690	1,289	23.5
12	23.6	2.9	8.4	2.0	32.1	254	346	841	1,474	24.0
15	21.5	3.3	10.0	2.4	17.4	265	325	698	1,145	24.0
18	23.6	4.3	14.9	3.5	17.1	268	350	718	1,350	24.0
21	21.5	4.6	12.8	3.0	11.8	264	349	772	1,363	25.0
24	23.6	5.7	15.3	3.6	12.5	231	308	834	1,305	24.0
Ave.	22.6	3.1	9.3	2.2	17.8	249	328	764	1,339	24.2
Control rats[2]		–	–	Trace	3.8	245	334	740	1,340	24.0

[1] The fat from one pig was fed to one male rat.

[2] This is the average of 29 rats fed a control diet of similar composition. The standard deviation was ± 4.0 p.p.m. of DDT.

There were no significant differences between male and female pigs in the storage of DDT, in daily gain, or in feed utilization when they received DDT-treated alfalfa hay (table 5).

TABLE 7

DDT content of rat fat and cumulative gain, food consumption and body length of rats fed swine bacon

ALFALFA HAY IN SWINE DIET	DDT					CUMULATIVE GAIN		FOOD CONSUMPTION		BODY LENGTH
	On alfalfa hay	In swine diet (dry basis)	In swine bacon [1]	In rat diet (dry basis)	In rat fat	After 8 wks.	After 14 wks.	After 8 wks.	After 14 wks.	
%	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*gm*	*gm*	*gm*	*gm*	*cm*
0	–	0	0.5	0.2	18.9	249	324	610	1,246	24.5
3	0	0	0.5	0.2	16.9	231	315	465	1,136	24.0
6	0	0	0.3	0.1	17.7	242	329	745	1,500	24.0
9	0	0	0.6	0.2	16.5	182	262	800	1,426	23.0
12	0	0	1.2	0.4	21.8	238	326	682	1,302	24.0
15	0	0	0.9	0.3	18.8	225	317	662	1,337	24.0
18	0	0	0.7	0.2	14.8	219	293	622	1,206	23.5
21	0	0	1.0	0.4	17.6	237	302	745	1,370	24.0
24	0	0	0.7	0.2	9.5	234	311	580	1,204	23.0
Ave.	0	0	0.7	0.2	16.9	228	309	657	1,303	23.7
3	21.5	0.7	2.3	0.8	12.0	230	294	831	1,413	25.5
6	23.6	1.4	4.0	1.4	9.4	212	308	675	1,284	22.5
9	21.5	2.0	4.1	1.4	15.0	254	341	734	1,302	24.0
12	23.6	2.9	6.9	2.4	12.7	233	305	567	1,035	24.0
15	21.5	3.3	9.1	3.2	24.0	251	318	927	1,683	24.0
18	23.6	4.3	11.2	3.9	28.8	213	303	711	1,287	24.5
21	21.5	4.6	7.4	2.6	18.4	221	298	594	1,167	24.5
24	23.6	5.7	8.4	3.0	11.6	207	281	630	1,202	22.5
Ave.	22.6	3.1	6.7	2.3	16.5	228	306	709	1,297	23.9
Control rats [2]		–	–	Trace	3.8	245	334	740	1,340	24.0

[1] The bacon from one pig was fed to one male rat.

[2] This is the average of 29 rats fed a control diet of similar composition. The standard deviation was ± 4.0 p.p.m. of DDT.

Histological studies of the swine liver showed that DDT did not cause any pathological changes.

After taking all of the data into consideration, it can be concluded that if swine are fed DDT-treated alfalfa contain-

ing 23 p.p.m. DDT for a normal fattening period, harmful effects will not result.

The effects of feeding the different levels of hay to the swine, disregarding the DDT, will be reported in another publication.

Effects of feeding swine tissues to rats

The DDT content of the raw fat, cured bacon and cured shoulder increased with each level of alfalfa hay in the swine

TABLE 8

DDT content of rat fat and cumulative gain, food consumption and body length of rats fed swine shoulder

ALFALFA HAY IN SWINE DIET	DDT					CUMULATIVE GAIN		FOOD CONSUMPTION		BODY LENGTH
	On alfalfa hay	In swine diet (dry basis)	In swine shoulder [1]	In rat diet (dry basis)	In rat fat	After 8 wks.	After 14 wks.	After 8 wks.	After 14 wks.	
%	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	gm	gm	gm	gm	cm
0	–	0	0.6	0.2	14.5	247	331	726	1,323	23.5
3	0	0	0.5	0.2	17.6	205	300	720	1,378	23.0
6	0	0	0.3	0.1	15.7	246	340	730	1,355	23.0
9	0	0	0.4	0.2	9.4	214	304	631	1,225	23.0
12	0	0	1.2	0.1	12.9	217	293	753	1,376	23.5
15	0	0	0.7	0.3	19.1	265	338	797	1,455	24.5
18	0	0	0.5	0.4	25.1	219	284	855	1,470	22.0
21	0	0	1.0	0.4	12.0	225	316	852	1,477	23.8
24	0	0	0.7	0.3	27.9	148	183	829	1,302	22.0
Ave.	0	0	0.6	0.2	17.1	221	299	766	1,373	23.0
3	21.5	0.7	2.3	0.9	6.0	241	319	794	1,475	23.0
6	23.6	1.4	3.0	1.2	11.4	239	324	774	1,423	24.5
9	21.5	2.0	2.6	1.1	10.9	269	361	779	1,385	25.0
12	23.6	2.9	4.9	2.0	26.1	237	317	793	1,480	25.0
15	21.5	3.3	8.7	3.5	28.7	198	284	818	1,306	22.5
18	23.6	4.3	7.9	3.2	35.9	247	301	802	1,427	23.0
21	21.5	4.6	6.9	2.7	29.7	250	326	800	1,452	23.0
24	23.6	5.7	6.0	2.4	30.7	202	270	804	1,354	23.0
Ave.	22.6	3.1	5.3	2.1	22.4	235	313	796	1,413	23.6
Control rats [2]		–	–	Trace	3.8	245	334	740	1,340	24.0

[1] The shoulder from one pig was fed to one male rat.

[2] This is the average of 29 rats fed a control diet of similar composition. The standard deviation was ± 4.0 p.p.m. of DDT.

diet. When the rats consumed these tissues, their fat contained relatively large amounts of DDT (tables 6, 7 and 8). There were no significant differences in the cumulative gain, food consumption or body length of the rats when they received raw fat, cured bacon or cured shoulder as part of their diet (tables 6, 7 and 8, fig. 2). The rats on this experiment gained at a rate comparable to that of control rats on a diet of similar composition (tables 6, 7, and 8).

There were no histological changes in the liver of any of the rats used.

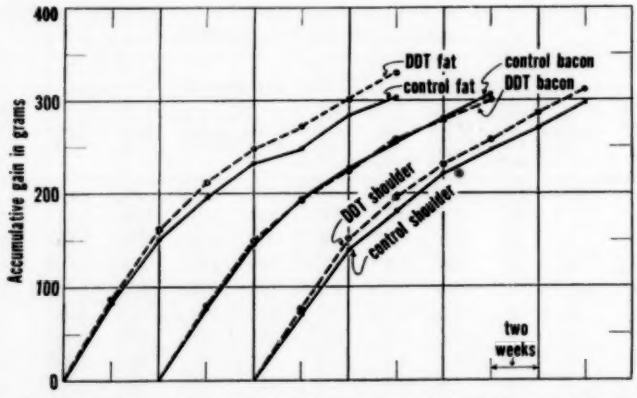

Fig. 2 Cumulative gain of rats fed fat, bacon and shoulder containing DDT.

These results show that fat, bacon and shoulder from pigs that had consumed DDT-treated alfalfa hay, dusted with DDT at a rate of two pounds per acre, were not toxic to rats when fed at a rate to supply 22% fat in their diet for a period of 14 weeks.

DISCUSSION

These studies show that DDT accumulates in the tissues of swine when they are fed alfalfa that has been treated with DDT, but that the DDT is not present in sufficient amounts to be toxic to the swine. These facts agree with the results of Arant ('48), Carter ('48) and Decker ('46).

Diets made up partly of swine tissues, to furnish not over 3.9 p.p.m. DDT, were not toxic to rats. Laug et al. ('50) also found no toxic effects in rats when they were fed less than 5 p.p.m. DDT in their diet for 20 weeks.

Some control swine and control rats had DDT in their tissues. This indicates that control animals should be housed separately from experimental animals and that every precaution should be taken to prevent contamination of control diets. It was found that most of the feeds in the control diets contained traces of DDT. Even dextrinized starch from several different sources contained small amounts; therefore it is almost impossible to make up a diet that is completely free of DDT. The contamination of control diets and the subsequent storage of DDT in the fat of control animals have also been reported by Laug et al. ('50).

For comparative purposes it is recommended that the DDT content of diets be reported on the dry basis.

SUMMARY

Twenty-two male and 22 female weanling pigs were fed control and DDT-treated alfalfa hay in their diets at the following percentage levels; zero, three, 6, 9, 12, 15, 18, 21, 24, 27, 30 and 33. The DDT-treated alfalfa was dusted in the field at the rate of two pounds of technical DDT per acre. There was a DDT residue of 23 p.p.m. (dry basis) on the harvested hay. The pigs were fed until they reached market weight (approximately 210 pounds). DDT did not affect the body weight gain, and there were no gross pathology or microscopic changes of the livers or kidneys of the pigs.

Weanling rats were fed raw fat, cured bacon and cured shoulder from the pigs for 14 weeks. The DDT in the rat diets (dry basis) averaged 2.2, 2.3 and 5.3 p.p.m. for the raw fat, cured bacon and cured shoulder diets, respectively. Under these experimental conditions, DDT did not influence either food consumption or gain in body weight. Autopsies were carried out on each animal and histological studies were con-

ducted on the livers and kidneys. All of the animals and tissues were normal.

ACKNOWLEDGMENTS

The authors wish to thank C. J. Sorenson, F. V. Lieberman and S. J. Snow for counting the insects on the alfalfa and applying the DDT to the alfalfa.

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FURTHER EVIDENCE AS TO THE POSSIBLE MECHANISM INVOLVED IN THE GROWTH-PROMOTING RESPONSES OBTAINED FROM ANTIBIOTICS

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(Received for publication July 22, 1953)

Elam et al. ('53) reported that penicillin greatly reduced the total number of *Clostridia* present in the intestinal tract and that small amounts of the antibiotic inhibited growth of the organisms *in vitro*.

Coates et al. ('51, '52) reported that chicks reared from one day to three weeks of age in quarters not previously used for chick experiments failed to show a growth response to penicillin, whereas such a response occurred in the old quarters. The chicks that were fed no antibiotic in the new quarters grew more rapidly than those fed the antibiotic in the old quarters. The authors concluded that the reason for the antibiotic response in the old quarters was the presence of an "infection" that made the chicks sensitive to the antibiotic.

Bird et al. ('51) reported that withdrawal of an antibiotic from the diet of chicks at 5 or 6 weeks of age caused a temporary retardation of growth when the birds were moved from one room to another at about the time of antibiotic withdrawal. These authors reported further that in later experiments in which the chicks were not moved, withdrawal of the antibiotic caused no retardation of growth.

Lillie et al. ('53) reported that the weight of chicks fed no antibiotic in new quarters was greater than that of chicks given 10 mg of aureomycin per kilogram of diet in old quar-

ters. They reported further that the growth-stimulating effect of the antibiotic was evident at one week of age in old quarters and increased in magnitude until the chicks were at least 4 weeks of age. The response to the antibiotic was considerably less in new quarters and was first evident at 4 weeks of age in one experiment and at two or three weeks in another. These workers also reported that when chicks were moved from new to old quarters at 4 weeks of age and deprived of the antibiotic, there was a temporary slump in growth rate, but that there was no such slump when the antibiotic was withdrawn from the diet of chicks that had been kept continuously in old quarters.

Hill et al. ('53) reported that growth response to penicillin was earlier and much greater in old quarters than in new ones. These workers reported further that bacteriological examination of the cecal microflora of representative birds revealed striking differences between the two animal houses in the relative numbers of various types of organisms and in the manner in which the latter responded to penicillin.

The object of the present experiments was to determine the effect of feeding antibiotics, *p*-chlorophenylarsonic acid and furazolidone(N-(5-nitro-2-furfurylidene)3-amino-2-oxazolidone) upon the weights of the birds and the clostridia population in the intestinal tracts in a clean environment.

EXPERIMENTAL

Data from two separate experiments are reported in the present study. The New Hampshire chicks used in these studies were obtained from hens that had been fed the diet described earlier by Elam et al. ('51b). The birds were housed in a clean room which had been washed daily for a period of two weeks before the beginning of the experiments. The room was washed daily during the entire experimental period. The birds were maintained in batteries with raised screen floors. The droppings were removed daily. Feed and water were supplied ad libitum and the chicks were weighed at weekly intervals during an experimental period of 10 weeks.

The basal diet used in this study consisted of 60.75% ground yellow corn, 35% soybean oil meal, 2% bone meal, 1.5% oyster flour and 0.5% NaCl. In addition, the diet was supplemented with 2 mg riboflavin, 5 mg calcium pantothenate, 12.5 mg niacin, 200 mg choline chloride, 100 mg manganese sulfate, 4,000 I.U. vitamin A (stable A), 600 I.C.U. vitamin D_3 and 13.6 μg vitamin B_{12} per pound of feed.

Level of supplements added

Antibiotics were fed at a level of 5 mg per pound of feed for aureomycin, bacitracin and chloromycetin, erythromycin was administered at a level of 2 mg per pound of feed and penicillin at a level of 2, 5, and 50 mg per pound of feed. In the second experiment, when the antibiotics were fed in combination, the total amount of antibiotic fed never exceeded 5 mg per pound of feed. Furazolidone and *p*-chlorophenylarsonic acid were administered at a level of 25 and 45 mg per pound, respectively. Penicillin was administered at a level of 1 mg per pound when fed in combination with furazolidone or *p*-chlorophenylarsonic acid, which were administered at a level of 12.5 and 22.5 mg per pound of feed, respectively.

Number of birds used

For the first experiment 75 chicks were randomized into three groups of 25 chicks each, and in the second experiment 300 chicks were randomized into 12 groups of 25 chicks each.

Bacteriological techniques used

Fecal samples were collected once a week and were prepared as described by Elam, Gee and Couch ('51a). A sulfite-glucose-iron agar, previously described by Wilson and Blair ('24) and Skinner and Baskin ('32), was used for determination of the anaerobes *(Clostridium)*. The one-to-10 dilution of the fecal samples was heat shocked at 65°C. for 30 minutes. The dilution plates made from these heat-shocked samples

were incubated anaerobically at 37°C. for 48 hours in Brewer anaerobic jars. All colonies on countable plates were counted. The colonies were then transferred to thioglycollate medium [1] and checked for their requirement for anaerobiosis. Virtually all colonies proved to be strict anaerobes and are therefore referred to throughout this paper as clostridia.

Statistical methods used

An analysis of variance for bacterial counts and chick weights was made, and statistical significance was determined from the F value (ratio of appropriate mean square values from an analysis of variance table).

TABLE 1

First experiment

The effect of penicillin and chloromycetin upon the weights of anaerobic microflora *(Clostridium)* of New Hampshire chicks at 10 weeks of age

GROUP	AVERAGE WEIGHT	TOTAL NUMBER OF CLOSTRIDIUM ISOLATES
	gm	*per gm of feces*
Control	1,217	2,875
Penicillin (2 mg/lb.)	1,287	2,560
Chloromycetin (5 mg/lb.)	1,182	2,700

RESULTS AND DISCUSSION

First experiment

In the first experiment the addition of penicillin or chloromycetin to the basal diet failed to produce a significant increase in the growth of the chicks (table 1). The administration of penicillin and chloromycetin also failed to influence the total number of fecal clostridia.

This is not in agreement with the work published earlier by Elam et al. ('53), where antibiotics increased the rate of gain and decreased the total number of clostridia per gram of feces. Two experiments were reported in the above paper

[1] Baltimore Biological Laboratories.

(Elam et al., '53), where the fecal material from the first test contained 45,480 and from the second 11,930 clostridia per gram, respectively. Penicillin failed to increase the growth of the birds in the experiment presently reported (table 1) and a low clostridia content of the feces was also evident (2,875) which was not significantly decreased by the addition

TABLE 2

Second experiment

The effect of antibiotics, a combination of antibiotics, furazolidone or *p*-chlorophenylarsonic acid and a combination of furazolidone and *p*-chlorophenylarsonic acid with penicillin upon the weights and anaerobic microflora *(Clostridium)* of New Hampshire chicks at 10 weeks of age.

GROUP	AVERAGE WEIGHT	TOTAL NUMBER OF CLOSTRIDIUM ISOLATES
	gm	*per gm of feces*
Control	1,215	1,540
Penicillin (2 mg/lb.)	1,217	795
Penicillin (5 mg/lb.)	1,307	355
Penicillin (50 mg/lb.)	1,292	520
Erythromycin (2 mg/lb.)	1,281	2,180
Bacitracin (5 mg/lb.)	1,218	1,810
Aureomycin (5 mg/lb.)	1,204	1,220
Penicillin (0.5 mg/lb.) + bacitracin (1.25 mg/lb.) + aureomycin (1.25 mg/lb.) + terramycin (1.25 mg/lb.)	1,265	950
Furazolidone (25 mg/lb.)	1,231	2,395
Furazolidone (12.5 mg/lb.) + penicillin (1 mg/lb.)	1,271	475
p-Chlorophenylarsonic acid (45 mg/lb.)	1,140	1,870
p-Chlorophenylarsonic acid (22.5 mg/lb.) + penicillin (1 mg/lb.)	1,190	975

to the basal diet of penicillin or chloromycetin. It should be pointed out that the laboratory and equipment in which the chicks were kept had been cleaned thoroughly and painted, and that the room was not occupied by chicks for approximately two and one-half months. Thus, it would appear that the chicks in the experiment presently reported were kept in a clean environment, and that the clostridia population of the intestinal tract was not sufficiently high so as to be significantly decreased by the feeding of antibiotics, as was true

in the earlier report (Elam et al., '53). Consequently the addition of an antibiotic to the basal diet in this test did not increase growth and had little if any effect on the clostridia content of the feces.

Second experiment

The growth obtained in the second experiment was about the same as that observed in the first experiment (tables 1 and 2). The growth of the birds was not increased by adding penicillin (at three different levels), aureomycin, bacitracin, erythromycin, furazolidone or *p*-chlorophenylarsonic acid to the diet. The feeding of furazolidone alone or in combination with penicillin or *p*-chlorophenylarsonic acid in combination with this antibiotic failed to produce an increase in growth. The administration of penicillin, aureomycin, bacitracin and terramycin in combination likewise failed to produce an increase in growth. The cleaning of the quarters in which the birds were maintained was the same as is described in experiment 1.

The number of clostridia per gram of feces was not decreased significantly in any of the instances in which antibiotics were fed singly or in combination. The feeding of furazolidone or *p*-chlorophenylarsonic acid alone or in combination with penicillin failed to produce a significant decrease in the total number of clostridia per gram of feces. Attention is directed to the fact that penicillin failed to increase growth or decrease the total number of clostridia even when fed at levels of 5 and 50 mg per pound of feed. The quantities of penicillin were decidedly higher than the amount which influenced the growth of chicks and the clostridia content of feces in the earlier report of Elam et al. ('53). The total number of clostridia was low in the control group of the second experiment as well as the first.

SUMMARY

Data from two separate experiments show that the oral administration of penicillin, erythromycin, aureomycin, ba-

citracin, chloromycetin, furazolidone or *p*-chlorophenylarsonic acid failed to stimulate the growth of chicks reared in clean quarters.

These supplements likewise failed to decrease the total number of clostridia present per gram of feces.

This is further evidence that antibiotics stimulate growth by reducing the total number of clostridia in the intestinal tract of the chick and are ineffective in stimulating growth in a clean environment where the clostridia population is low.

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INFLUENCE OF DIETARY AMINO ACID SUPPLEMENTS ON THE FREE AMINO ACIDS IN THE BLOOD PLASMA OF CHICKS

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(Received for publication July 24, 1953)

In a survey of the effect of the dietary level of vitamins on the free amino acids in the plasma of chicks, Richardson, Blaylock and Lyman ('53) found that some of the free amino acids were present in smaller amounts with a low level of intake of some vitamins and higher with a low level of other vitamins. Heir ('47) investigated the effect of feeding an excess of several amino acids singly to dogs and found that the level of the corresponding amino acid in the plasma increased. In most cases a high level of one amino acid was associated with a drop in others, but there were a few instances when an increase in one was associated with an increase in another. The reader is referred to the paper by Heir ('47) for a review of earlier studies of the effect of single animo acids on the level of blood amino nitrogen.

Blaylock and Richardson ('50) investigated peanut meal and mixtures of soybean and peanut meals as sources of protein for the growth of baby chicks. When the diet contained peanut meal as the only protein concentrate, the addition of 0.4% of DL-methionine depressed the rate of growth. The addition of 0.2% of L-lysine increased the rate of growth, but the final weights were still below those of chicks of the

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same age and breed receiving an adequate diet. The chicks grew rapidly when the peanut meal diet was supplemented with both methionine and lysine. Since supplementing with methionine and lysine changed the rate of growth of chicks, it was of interest to determine whether or not the free amino acids of the plasma were changed by the addition of these amino acids to the diet. It was of interest also to determine the influence of an excess of a single amino acid on the free amino acids of the plasma when the diet was adequate in protein. This report describes the results of studies of the effect on the concentrations of free arginine, lysine, methionine, tryptophan and valine in the plasma: (1) when lysine and methionine were fed as supplements to a peanut meal diet; and (2) when an excess of methionine and of valine were fed as supplements to a diet that was adequate in protein.

EXPERIMENTAL

The procedures used for the care of the chicks, for taking the blood samples, for the preparation of the protein-free filtrates, and for the microbiological assays were the same as those described in detail in an earlier report by Richardson et al. ('53).

A basal diet composed chiefly of milo and peanut meal was used to study the effect of a dietary deficiency of lysine and of methionine on the concentration of the free amino acids in the plasma. The composition of the peanut meal diet is given in table 1. A semisynthetic diet was used to study the effect of a dietary excess of methionine and of valine on the free amino acids in the plasma. A soybean meal diet was used for the purpose of comparison. The compositions of the semisynthetic and soybean meal diets were given in an earlier report (Richardson et al., '53) as those of diets 1 and 2, respectively.

Day-old mixed sex crossbred chicks (Nichols New Hampshire males $\times$ crossbred females) obtained from a commercial hatchery were used. The total numbers of chicks in a group are given in tables 2 and 3. Two series of experiments were

run at a different time for each test. The chicks in a group were divided into two subgroups at the end of the experimental period so that the average weight of each subgroup was the same as that of the original group. An equal amount of blood was drawn by heart puncture from each chick in a subgroup and pooled. The total amount of blood collected for a sample was approximately 80 ml. Thus 4 samples of plasma were obtained for each diet, and the free amino acids in the protein-free filtrates were determined by microbiological assay. The values were analyzed statistically by the "t" test.

TABLE 1

Composition of peanut meal diet

CONSTITUENTS	AMOUNT	VITAMINS PER 100 GM OF DIET	AMOUNT
	%		*mg*
Ground milo	64.5	Alpha-tocopherol acetate	2.5
Peanut meal	32.0	Thiamine hydrochloride	0.4
Steamed bone meal	2.0	Riboflavin	0.4
NaCl	0.5	Pyridoxine	0.4
$MnSO_4 \cdot H_2O$	0.04	Calcium pantothenate	2.0
Wesson oil	0.2	Niacin	5.0
Fish liver oil	0.8	Vitamin B_{12}	0.001
Choline chloride	0.2	Menadione	2.5

Amino acids in the plasma of chicks and those calculated in the diet

The calculated amounts of the amino acids in the semisynthetic (SS), peanut meal (PNM) and soybean meal (SBM) diets, and the corresponding amounts of the free amino acids in the plasma from chicks which received each diet, are given in table 2. The concentration of a free amino acid in the plasma was highest, with two exceptions, when it was highest in the diet. Arginine was slightly lower in the soybean meal than in the semisynthetic diet, but there were 94 μg/ml of arginine in the plasma from chicks which received the soybean meal diet, compared with 49 μg in the plasma of those which received the semisynthetic diet. The semisynthetic contained more tryptophan than either the peanut meal or soy-

TABLE 2

The free amino acids in the plasma of chicks and the calculated amounts in diets

DIET	NO. OF CHICKS	AVE. WT. OF CHICKS [1]	PROTEIN IN DIET	ARGININE		LYSINE		METHIONINE		TRYPTOPHAN		VALINE	
				diet	plasma	diet	plasma	diet	plasma	diet	plasma	diet	plasma
		gm	%	%	*μg/ml*	%	*μg/ml*	%	*μg/ml*	%	*μg/ml*	%	*μg/ml*
SS	84	515	23.7	1.261	49	1.423	110	0.566	48	0.291	62	1.409	191
PNM	26	364	20.0	1.682	98	0.608	15	0.267	29	0.212	64	0.937	136
SBM	39	515	20.0	1.229	94	0.975	69	0.321	34	0.265	76	1.038	176

[1] Experimental period, 6 weeks.

TABLE 3

Influence of dietary amino acids on the free amino acids in the blood plasma of chicks
(Groups 2, 3 and 4 were compared with group 1; groups 6 and 7 with group 5)

GROUP NO.	SUPPLEMENT	NO. OF CHICKS	WT. OF CHICK		FREE AMINO ACIDS IN THE PLASMA — AVE. μG/ML									
					Arginine		Lysine		Methionine		Tryptophan		Valine	
			Ave.	Diff.	Ave.	Diff.	Ave.	Diff.	Ave.	Diff.	Ave.	Diff.	Ave.	Diff.
	A. Methionine and lysine as supplements to a peanut meal diet (PNM)[1]													
1	None	26	364		98		15		29		64		136	
2	DL-Methionine 0.4%	26	330	— 34	87	— 11	12	— 3	61	32**	61	— 3	118	— 18**
3	L-Lysine 0.2%	25	443	79** [2]	116	18*	33	18**	28	— 1	63	— 1	155	19**
4	DL-Methionine 0.4% L-Lysine 0.2%	24	567	203**	91	— 7	14	— 1	64	35**	60	— 4	115	— 21**
	B. Excess methionine and valine as supplements to an adequate diet (semisynthetic — SS)[3]													
5	None	20	1,108		67		108		69		78		220	
6	DL-Methionine 0.5%	20	1,144		57	— 10**	78	— 30**	110	41**	83	5	174	— 46**
7	DL-Valine 0.5%	20	1,107		65	— 2	99	— 9	71	2	78	0	292	72*

[1] Experimental period was 6 weeks.
[2] * Significant at 5% level by use of "t" test.
** Significant at 1% level by use of "t" test.
[3] Chicks had received the basal diet for 8 weeks and amino acid supplements for one week. Weight equals average final weight.

bean meal diet, but the plasma from the chicks which received this diet contained slightly less tryptophan than that from those which received the other two diets. The free lysine in the plasma of chicks receiving the peanut meal diet was very low. There was an average of 15 μg/ml, compared with 69 and 110 μg/ml in the plasma of those receiving the soybean and semisynthetic diets, respectively.

Free amino acids in the plasma when the diet was adequate and when it was deficient in lysine or methionine or both

Since the basal diet containing peanut meal as the sole source of protein is deficient in both methionine and lysine, it was used to determine the effect of various amounts of dietary lysine and methionine on the free amino acids in the plasma. Four groups of chicks were used in each of the two experiments. There were 16 chicks per group in the first and 10 per group in the second experiment. Group 1 received the basal diet; group 2 received a supplement of 0.4% DL-methionine; group 3 received 0.2% of L-lysine; and group 4 received both amino acids. The experimental period was 6 weeks.

The average weight of the chicks and concentrations of free arginine, lysine, methionine, tryptophan and valine in the plasma are summarized in table 3 (section A). The observations on growth were essentially the same as those reported previously. The addition of lysine (group 3) alone increased the average weight of the chicks from 364 (group 1) to 443 gm, while the addition of methionine (group 2) alone decreased the average weight to 330 gm. When both amino acids (group 4) were added, the average weight was 567 gm. These data show that when the diet was low in lysine, the addition of methionine depressed the rate of growth. However, the same amount of methionine had no depressive effect when the diet was adequate in lysine. When methionine was added to the diet, the concentration of lysine in the plasma of the chicks was essentially the same or slightly less, either

with or without lysine, than it was in the plasma of those which received the basal diet. The addition of methionine depressed growth probably because it increased the deficiency of lysine in a diet that was already low in this amino acid.

A dietary supplement of a single amino acid always increased the concentration of the corresponding free amino acid in the plasma. The concentrations of some of the other amino acids were changed by one supplement but not by another supplement. None of the supplements changed the concentration of tryptophan. Supplementing with methionine alone increased the concentration of methionine and decreased the concentration of arginine, lysine and valine, but the decrease for arginine and lysine was not significant. Lysine alone increased the concentration of arginine, lysine and valine, but it did not change that of methionine. When both amino acids were added, the concentrations of the 5 amino acids in the plasma were essentially the same as when methionine was added alone. Thus it appears that methionine has a greater influence on some of the amino acids in plasma than lysine.

Influence of excess dietary methionine and valine on free amino acids in the plasma

The effect of an excess of methionine and of valine in the diet on the concentration of free amino acids in the plasma of chicks was investigated. In this study the amino acids were added to a diet that was adequate in protein for a rapid rate of growth. Chicks which had received the semisynthetic diet (SS; diet 1, Richardson et al., '53) for 8 weeks were used for this purpose. They were divided into three groups. One, group 5, was continued on the basal diet; the second, group 6, received a supplement of 0.5% of DL-methionine; and the third, group 7, received 0.5% of DL-valine. There were 10 chicks per group in each of two experiments. The amino acids were supplied for one week. Blood samples were taken and treated as described previously and there were two separate experiments run at different times as in the case of the

other studies. The results are summarized in table 3 (section B). As was the case when the diet was deficient in methionine, an excess of methionine decreased the concentration of free arginine, lysine and valine and it increased the concentration of methionine. An excess of dietary valine had no influence on the concentration of any of the 5 amino acids in the plasma that were studied, with the exception of valine; it increased the concentration of valine. These data show that the amount of methionine in the diet in general affects the concentration of certain amino acids in the plasma more than the amount of either lysine or valine.

SUMMARY

The depression in the rate of growth that was produced by supplementing a peanut meal diet with methionine alone appeared to be associated with a deficiency of lysine. When adequate lysine was present, the addition of methionine did not depress the rate of growth. The addition of DL-methionine to the diet of chicks had essentially the same effect on certain free amino acids in the plasma regardless of whether the diet was adequate or deficient in methionine. It decreased the concentration of arginine, lysine and valine under both conditions. Except for arginine and lysine, the differences were highly significant when the diet was deficient. Methionine increased the concentration of methionine in the plasma, but it had no effect on the concentration of tryptophan. The addition of L-lysine to the diet increased the concentration of free arginine, lysine and valine in the plasma. It had no effect on the concentration of methionine or tryptophan under these conditions. When both lysine and methionine were added to the deficient diet, the concentrations of free amino acids in the plasma were essentially the same as when methionine was added alone. The amount of dietary methionine appeared to have a much greater influence on the concentration of some of the amino acids in the plasma which were studied than the amount of either lysine or valine. The addition of valine to a diet that was adequate in protein increased the

concentration of free valine in the plasma. It had no influence on the concentration of the other amino acids which were studied.

ACKNOWLEDGMENTS

The authors are indebted to Merck and Company, through the courtesy of Dr. H. Draper, for the crystalline vitamins, and to Lederle Laboratories, through the courtesy of Dr. T. H. Jukes, for the folic acid.

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INFLUENCE OF VARIOUS CARBOHYDRATES ON THE UTILIZATION OF LOW PROTEIN RATIONS BY THE WHITE RAT

II. COMPARISON OF SEVERAL PROTEINS AND CARBOHYDRATES. GROWTH AND LIVER FAT [1]

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(Received for publication July 27, 1953)

In a previous paper, Harper and Katayama ('53) reported that 9% casein diets support better growth of rats when sucrose is replaced, as the source of dietary carbohydrate, by either cornstarch or dextrin. An effect of the type of dietary carbohydrate on liver fat deposition in rats fed low-protein diets has also been reported, and it has been shown that the accumulation of fat in the livers of rats which are fed a 9% casein-sucrose diet containing choline is reduced when sucrose is replaced by dextrin (Litwack, Hankes and Elvehjem, '52; Harper, Monson, Benton and Elvehjem, '53b). Apparently dextrin acts in both instances by permitting more efficient utilization of the protein in these low-protein diets.

Since Litwack, Williams, Chen and Elvehjem ('52) have shown that the xanthine oxidase activity of rat liver is positively correlated with the amount of protein in the diet, and since the growth and liver fat studies cited above indicate that dextrin acts by improving protein utilization, replacement of

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. This work was supported in part by grants from Swift and Company, Chicago; the Gelatin Research Society of America, Inc., New York City; and the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.

sucrose by dextrin in low-protein diets might be expected to cause an increase in liver xanthine oxidase activity.

In the present paper experimental evidence is presented which extends previous reports on the influence of dietary carbohydrates on both growth and liver fat deposition, and an experiment on the effect of dietary carbohydrate on liver xanthine oxidase activity is included.

EXPERIMENTAL

Male weanling rats of the Sprague-Dawley strain weighing from 40 to 50 gm were used throughout these studies. They were divided into similar groups of 6 animals each and were maintained in individual cages with raised screen bottoms. Unless otherwise noted, the animals were fed ad libitum and each animal was weighed weekly during the experimental periods of from two to 12 weeks.

The composition of the basal diet was as follows: sucrose, 81.6; casein, 9.0; DL-methionine, 0.3; corn oil, 5.0; salts 4 (Hegsted, Mills, Elvehjem and Hart, '41), 4.0; and choline chloride, 0.13%. Vitamins were included to provide, in milligrams per 100 gm of ration, thiamine hydrochloride, 0.5; riboflavin, 0.5; niacin, 1.0; calcium pantothenate, 2.0; pyridoxine, 0.25; biotin, 0.01; folic acid, 0.02; vitamin B_{12}, 0.002; and inositol, 10.0. Two drops of halibut liver oil, fortified to provide vitamin A, 1,000 I.U.; vitamin D, 10 I.U.; 2-methyl,1-4 naphthoquinone, 0.04 mg and α-tocopherol, 0.8 mg, were administered weekly.

Supplements, including increases in protein, were included in the diet at the expense of carbohydrate. The carbohydrate component of each diet is listed in the tables, which present our experimental results. In experiment 5, which was of only two weeks' duration, 0.1% DL-tryptophan was included in order to insure more uniform growth. Casein was omitted from the diets in experiments in which other proteins were tested, except for experiment 6, in which 0.23% casein was used as a carrier for vitamins. The composition of the amino acid

mixture used in experiment 6 is listed in table 1 and was calculated to be equivalent to 9% casein (12.4% N). The analysis presented by Block and Bolling ('51) was used as the basis for the calculations, except that the amount of L-proline, which was non-essential and costly, was reduced.

The various proteins and carbohydrates, except dextrin, were commercial products. The casein was extracted three times with boiling alcohol. The term "dextrin" used through-

TABLE 1

Composition of amino acid mixture equivalent to 9% casein

AMINO ACID	% IN DIET	COMMENTS
L-arginine·HCl	0.39	
L-histidine·HCl·H_2O	0.33	
L-lysine·HCl	0.87	
L-tyrosine	0.49	
DL-tryptophan	0.12	D = 3/4 L for rats (Oesterling and Rose, '52)
DL-phenylalanine	0.48	D = L
L-cystine	0.03	
DL-methionine	0.27	D = L
DL-threonine	0.68	D not utilized
DL-serine	0.52	
DL-leucine	1.52	D not utilized
DL-isoleucine	1.09	D not utilized
DL-valine	1.16	D not utilized
L-glutamic acid	1.75	
DL-aspartic acid	0.53	
Glycine	0.16	
DL-alanine	0.25	
L-proline	0.30	

out this paper refers to the product obtained by heating moist cornstarch in an autoclave at 15 pounds' pressure for three hours. This material gave a blue color with iodine. Dextrin A was prepared by heating dry cornstarch in an oven at 145°C. for 10 hours. Dextrin M was a commercial product. The last two gave red-violet colors with iodine, indicating the presence of shorter chain molecules.

The paired-feeding experiment was conducted under exactly the same physical conditions as the ad libitum trials.

The rats in the sucrose group were allowed to eat ad libitum and each of those fed dextrin was given, every second day, an amount of food equivalent to that consumed by its control in the sucrose group. Spillage was carefully measured and corrected for at each feeding.

At the termination of the experimental period each rat was killed by a blow on the head, was decapitated and bled, and the liver was excised for the determination of fat. Fat was determined by ether extraction of the dried and ground liver (Hawk and Elvehjem, '53).

For xanthine oxidase determinations, the animals were killed as described above. Each liver, immediately after removal, was chilled in ice and blotted free of moisture. A weighed portion of each liver was homogenized with 5 volumes of ice-cold 0.039 M sodium potassium phosphate buffer (pH 7.3). The xanthine oxidase activity of the homogenates was determined by the method of Axelrod and Elvehjem ('41).

Nitrogen was determined by the Kjeldahl method. Mercuric oxide was used as the catalyst.

Histological studies of the livers of animals from experiment 5 will be reported separately.

RESULTS

Each experiment is reported individually because the absolute values for similar groups vary from experiment to experiment even though the relative values are consistent. A part of the variation observed can probably be accounted for by variations in the nitrogen content of different lots of casein, and by variations in the ages of the animals at the end of the different experiments. Two control groups, one fed the sucrose and the other the dextrin basal diet, were included in each experiment to permit accurate comparisons.

Carbohydrates

The results of experiments designed to determine the effect of various carbohydrates on growth and liver fat deposition in rats fed low-casein diets are presented in table 2. In every

TABLE 2

Effect of various carbohydrates on growth and liver fat of rats fed low casein diets[1]

EXP. NO.	GROUP NO.	TYPE OF DIET	RATE OF GAIN	LIVER FAT	
				dry weight	wet weight
			gm/wk.	%	%
1	1	9% casein-sucrose	15.5 ± 1.7[2]	25.9 ± 2.1[2]	8.4 ± 0.8[2]
	2	9% casein-dextrin	34.4 ± 0.4	19.2 ± 1.5	6.0 ± 0.5
	3	9% casein-sucrose + 10% lactose	20.9 ± 0.7	24.4 ± 1.6	7.6 ± 0.6
2	4	9% casein-sucrose	11.3 ± 1.6	23.2 ± 1.8	7.3 ± 0.7
	5	11.2% casein-sucrose	22.4 ± 2.0	13.8 ± 0.9	4.1 ± 0.3
	6	13% casein-sucrose	31.1 ± 2.4	9.8 ± 0.5	2.8 ± 0.2
	7	9% casein-dextrin	31.9 ± 1.5	22.6 ± 2.0	7.0 ± 0.7
	8	11% casein-dextrin	39.7 ± 0.7	8.9 ± 1.1	2.8 ± 0.3
	9	9% casein-cerelose	23.1 ± 1.0	15.9 ± 0.4	4.5 ± 0.1
	10	9% casein-sucrose + 10% lactose	15.7 ± 0.9	26.1 ± 2.8	8.5 ± 1.1
	11	9% casein-sucrose 5% ea. gum acacia, cellulose	18.6 ± 1.2	20.3 ± 1.9	6.3 ± 0.7
3	12	9% casein-sucrose	15.0 ± 1.2	32.9 ± 2.3	11.1 ± 1.0
	13	9% casein-sucrose:dextrin 3:1	19.2 ± 1.9	36.0 ± 2.9	12.9 ± 1.3
	14	9% casein-sucrose:dextrin 1:1	24.6 ± 1.4	32.8 ± 4.5	11.4 ± 2.2
	15	9% casein-sucrose:dextrin 1:3	27.7 ± 2.3	30.0 ± 3.3	10.1 ± 1.5
	16	9% casein-dextrin	31.4 ± 2.0	...	...
	17	9% casein-dextrin A[3]	25.2 ± 2.1	...	...
	18	9% casein-dextrin M[3]	25.9 ± 1.0	18.0 ± 1.8	5.3 ± 0.5
	19	9% casein-glucose	17.8 ± 0.8	19.3 ± 0.6	6.0 ± 0.2
4	20	9% casein-sucrose	17.8 ± 1.3	26.8 ± 1.9	8.7 ± 0.8
	21	9% casein-dextrin	34.3 ± 1.8	16.3 ± 1.0	4.8 ± 0.3
	22	9% casein-dextrin:cerelose 3:1	34.4 ± 1.7	17.6 ± 0.5	5.1 ± 0.1
	23	9% casein-dextrin:levulose 3:1	32.6 ± 2.1	22.4 ± 2.7	7.1 ± 1.0
	24	9% casein-dextrin:sucrose 3:1	31.6 ± 1.5	19.1 ± 1.2	5.8 ± 0.4
	25	9% casein-cerelose	22.9 ± 0.9	18.7 ± 1.1	5.6 ± 0.3
	26	9% casein-cerelose:levulose 3:1	26.2 ± 1.5	22.2 ± 2.6	7.0 ± 1.0
	27	9% casein-cerelose:dextrin 3:1	27.8 ± 0.7	17.2 ± 2.1	5.1 ± 0.7
	28	9% casein-cerelose:sucrose 3:1	23.7 ± 1.1	27.1 ± 2.5	8.3 ± 1.0
	29	9% casein-cerelose:sucrose 1:1	21.2 ± 1.6	27.3 ± 2.4	8.2 ± 0.9
5	30	9% casein-sucrose	19.7 ± 1.9	34.1 ± 2.9	10.8 ± 1.2
	31	9% casein-dextrin	36.2 ± 0.6	15.4 ± 1.2	4.1 ± 0.4
	32	9% casein-cerelose	28.0 ± 1.3	15.0 ± 0.4	4.1 ± 0.2
	33	9% casein-levulose	13.8 ± 1.2	29.6 ± 2.6	9.5 ± 1.0

[1] Experiment 1 was of 5 weeks' duration; 2 and 3, 4 weeks'; 4, three weeks' and 5, two weeks'.

[2] Mean ± standard error of the mean for 6 rats.

[3] See text.

experiment with 9% casein diets the groups fed dextrin grew at between double and triple the rate of those fed sucrose (groups 1 and 2, 4 and 7, 12 and 16, 20 and 21, 30 and 31). In experiment 2, among the sucrose groups, only group 6, which received 13% casein, grew as well as the 9% casein-dextrin control (group 7). When only 25% of the carbohydrate was supplied as sucrose (group 15), the rate of growth was reduced slightly below that of the dextrin group. Dextrins A and M, the shorter chain products, did not support quite as good growth (groups 17 and 18) as the dextrin used routinely.

When cerelose (glucose monohydrate) was used as the carbohydrate (groups 9, 25, 32), it supported a rate of growth intermediate between that observed with sucrose and that observed with dextrin. The inclusion of 10% lactose (groups 3 and 10) or 10% bulk (group 11) in the basal ration increased growth slightly. The use of fructose to supply 25% of the carbohydrate with dextrin or cerelose (groups 23 and 26) had little effect on growth but when it was fed as the sole source of carbohydrate growth was slightly depressed (experiment 5).

In every experiment liver fat deposition [2] was highest (25 to 39%) in groups which were fed the sucrose basal diet (groups 1, 4, 12, 20, 30) and in every case except one (group 7) least fat (16 to 22%) accumulated in the livers of animals fed dextrin. Even in the experiment in which the exception occurred with 9% casein, a difference between the sucrose and the dextrin groups was observed with 11% casein. Liver fat depositions in the glucose and the dextrin groups were usually similar. Fructose, like sucrose, induced greater liver fat deposition. In experiment 4, in which only 25% of the carbohydrate was supplied as fructose (groups 23 and 26), liver fat was slightly elevated over that of the comparable groups which received no fructose. In experiment 5, when all of the carbohydrate was provided as fructose, liver fat deposition was as high as that of the sucrose group.

[2] All liver fat values cited in the text are based on the dry weight of liver.

TABLE 3

Growth and liver fat of rats receiving sucrose or dextrin in various low-protein diets[1]

EXP. NO.	GROUP NO.	TYPE OF DIET	RATE OF GAIN	LIVER FAT	
			gm/wk.	%	%
6	34	9% casein-sucrose	19.1 ± 1.8[2]	26.8 ± 3.0[2]	8.4 ± 1.1[2]
	35	9% casein-dextrin	36.5 ± 1.4	18.2 ± 1.4	5.3 ± 0.5
	36	Amino acids ≅ 9% casein-sucrose[3]	20.6 ± 0.8	23.4 ± 1.0	7.2 ± 0.3
	37	Amino acids ≅ 9% casein-dextrin[3]	29.6 ± 1.6	18.7 ± 1.8	5.4 ± 0.5
	38	15% wheat gluten + 0.7% lysine-sucrose	19.0 ± 2.1	18.2 ± 1.3	5.5 ± 0.4
	39	15% wheat gluten + 0.7% lysine-dextrin	35.5 ± 2.2	15.8 ± 1.1	4.4 ± 0.3
	40	9% egg albumin-sucrose	13.7 ± 1.2	23.9 ± 2.5	7.4 ± 1.0
	41	9% egg albumin-dextrin	24.0 ± 1.4	26.6 ± 4.3	8.5 ± 1.7
7	42	9% casein-sucrose	15.0 ± 1.2	39.9 ± 3.9	14.7 ± 2.7
	43	9% casein-dextrin	31.3 ± 3.0	24.9 ± 2.6	7.8 ± 0.9
	44	9% casein + 6% gelatin-sucrose	17.5 ± 2.0	16.3 ± 2.2	4.8 ± 0.7
	45	9% casein + 6% zein-sucrose	22.8 ± 1.5	27.3 ± 2.3	8.8 ± 0.9
	46	9% egg albumin-sucrose	13.8 ± 0.7	28.7 ± 2.2	9.2 ± 0.9
	47	9% egg albumin + 0.5% lysine-sucrose	11.9 ± 1.3	33.7 ± 2.5	11.8 ± 1.1
	48	9% fibrin-sucrose	14.3 ± 0.8	16.1 ± 1.0	4.9 ± 0.3
	49	9% fibrin-dextrin	32.2 ± 3.2	17.4 ± 1.0	5.2 ± 0.5

[1] Experiment 6 was of 4 weeks' duration; 7, three weeks'.

[2] Mean ± standard error of the mean for 6 rats.

[3] See table 1.

Proteins

The results of experiments designed to compare the effects of sucrose and dextrin on the growth and liver fat deposition of rats fed some other proteins are presented in table 3. Growth differences between groups of rats fed sucrose and those fed dextrin were observed with three other proteins: wheat gluten supplemented with lysine (groups 38 and 39), egg albumin (groups 40 and 41) and fibrin (groups 48 and 49). A growth difference was also observed when 9% casein was replaced by its equivalent in amino acids (groups 36 and 37). In each instance dextrin supported much better growth than sucrose.

TABLE 4

Influence of paired feeding on growth and liver fat of rats receiving sucrose or dextrin in 9% casein diets[1]

	GAIN/WK.	DAILY FOOD INTAKE	LIVER FAT	
			Dry wt.	Wet wt.
	gm	*gm*	%	%
9% casein-sucrose	15.0 ± 1.2[2]	8.8 ± 0.6[2]	32.9 ± 2.3[2]	11.1 ± 1.0[2]
9% casein-dextrin	16.5 ± 1.3	8.8 ± 0.6	16.3 ± 2.1	4.8 ± 0.7

[1] Experiment was continued 4 weeks.

[2] Mean ± standard error of the mean for 6 rats.

The effect of dextrin in lowering liver fat was demonstrable only with the casein and the amino acid diets. With the wheat gluten and the fibrin diets relatively low liver fat was found, regardless of the type of dietary carbohydrate. With the albumin diet, high values were obtained even in the presence of dextrin (groups 40 and 41) and the addition of lysine to the albumin-sucrose diet (group 47) did not affect liver fat deposition.

The addition of gelatin or zein (groups 44 and 45) to the casein diet brought about reductions in liver fat. Zein was less effective than gelatin. The results given in table 2 (groups 4 to 8) show that a reduction in liver fat also occurred when the casein in the diet was increased.

In table 4 the results of a paired-feeding experiment are presented. In this experiment, in which the food intake of the dextrin group was kept down to that of the sucrose group, only a slight growth difference between the two groups was observed; however, less fat was found in the livers of the animals fed dextrin.

The results of a 12-week experiment are presented in table 5. The values for liver fat at 4 weeks were taken from group 18, which was fed dextrin, and from the sucrose group of the paired-feeding experiment, experiments which were run concurrently. The growth rate of rats receiving the 9% casein-sucrose ration increased, while that of animals fed dextrin decreased, over the 12-week period. Although the liver fat

TABLE 5

Influence of time on growth and liver fat of rats receiving sucrose or dextrin in 9% casein diets

	GROWTH		LIVER FAT (DRY WT.)	
	4 wks.	12 wks.	4 wks.	12 wks.
	gm/wk.	*gm/wk.*	%	%
9% casein-sucrose	14.5 ± 0.9 [1]	19.1 ± 0.8 [1]	32.9 ± 2.3 [1]	14.4 ± 1.0 [1]
9% casein-dextrin	31.4 ± 2.0	25.6 ± 0.9	18.0 ± 1.8	14.1 ± 0.8

[1] Mean ± standard error of the mean for 6 rats.

of the sucrose animals was high (32.9%) after 4 weeks, by 12 weeks it had dropped to 14%, the same as that of the dextrin group.

Values for the xanthine oxidase activity of liver homogenates from animals fed the sucrose and the dextrin diets were 30.0 ± 5.7 and 50.5 ± 2.5 µl of oxygen per gram of liver per hour, respectively. Although the values for both groups are relatively low (the normal value for rats of comparable age fed 18% casein is 100 to 130), a substantial difference between the two groups was observed.

DISCUSSION

Growth

The results of the growth study extend the observations of Harper and Katayama ('53). Since many factors (strain

of rats, sex ratio of groups, vitamin and mineral content of the diets, environment) were altered in the latter of the two studies, the differences observed are not surprising. In the present experiments a somewhat greater growth rate was observed throughout than in the previous work, and slightly more casein was required in the sucrose diet to support growth equal to that obtained with 9% casein and dextrin. The relative values, however, are in good agreement, and it is evident that dextrin permits much better growth of rats fed low-casein diets ad libitum than does sucrose.

The results obtained with wheat gluten, fibrin and albumin (table 2) indicate that this phenomenon is not restricted to low-casein diets. The excellent growth obtained with 15% wheat gluten supplemented with lysine when dextrin was used as the carbohydrate is of interest in view of recent reports of the value of amino acid supplementation of wheat proteins (Sure, '52, '53) and rice proteins (Pecora and Hundley, '51). Since Pecora ('53) has also shown that antibiotics stimulate the growth of animals fed rice proteins supplemented with lysine and threonine, it would be of interest to know whether animals fed wheat proteins might respond similarly.

The intermediate growth observed with cerelose as the carbohydrate suggests that it too permits better utilization of protein than does sucrose. Similar results have been observed in studies with chicks (Monson, Dietrich and Elvehjem, '50). The behavior of fructose paralleled that of sucrose.

The results of the paired-feeding experiment indicate that the more efficient utilization of low-protein diets containing dextrin (Harper and Katayama, '53) is associated with increased food consumption. However, failure to demonstrate the effect of dextrin on growth in such an experiment may be associated with the non-physiological conditions of alternate starvation and immoderate eating.

The 12-week study (table 4) shows that, although a substantial weight difference between rats fed sucrose and those fed dextrin was evident even after 12 weeks, the rates of

growth were beginning to converge. This convergence parellels the decreased protein requirement of maturing animals.

It has been suggested previously that at least part of the growth response is related to the rate of digestion and absorption of the carbohydrate and protein components of the diets. The lower growth obtained when shorter-chain dextrins were fed (groups 17 and 18) suggests that the size of the molecule of the carbohydrate in the diet is of significance. The growth reduction observed when casein in the dextrin diet was replaced by amino acids suggests that the rate of release or absorption of amino acids from casein is also important. However, since dextrin supported much better growth than sucrose even when casein was replaced by amino acids, it is apparent that intact protein is not essential for this effect. Concurrent absorption of carbohydrate and amino acids, which probably occurs over a longer period of time with dextrin in the diet, may reduce the loss of amino acids through deamination (Geiger, '51). Since it is well-known that dextrin and sucrose produce different types of intestinal flora, the effect observed may be due, at least in part, to these changes.

The growth rates of the groups fed amino acids are of interest in their own right, in view of the observations of Maddy and Swift ('52) on the failure of amino acids to support as good growth as intact casein. With sucrose as the dietary carbohydrate, amino acids simulating 9% casein supported as good growth as did casein. With dextrin as the dietary carbohydrate, the growth of the amino acid group was somewhat less than that of the comparable casein group; nevertheless the average growth of the rats (29.6 gm/wk.) which received amino acids equivalent to only 9% casein was equal to that obtained by Maddy and Swift ('52), using sucrose with 23.25% amino acids plus an antibiotic. The only protein supplied in the amino acid rations used in this study was 0.23% casein, which was used as a carrier for the vitamins. Whether the good growth of rats fed the amino acid diets was merely the result of ideal experimental conditions

at the time, or whether it may be attributable to some factor related to the small amount of casein, remains to be determined. Since the casein could scarcely supply a significant amount of amino acids, the amino acid analysis of casein presented by Block and Bolling ('51) is evidently quite accurate in terms of biological utilization.

Liver fat

The observation that less fat was deposited in the livers of rats fed 9% casein diets when sucrose was replaced by dextrin confirms previous observations (Litwack et al., '52; Harper et al., '53b). The reduction in liver fat observed when cerelose was fed and the high liver fat when fructose was fed have not been reported previously. Since a reduction also occurred when more protein was added to the casein-sucrose basal diet, the accumulation of liver fat is apparently caused by the low level of protein. However, the suggestion that sucrose and fructose may be metabolized in a manner that favors fat formation (see Lamb, '50, for references) merits further investigation. Nevertheless, the reduction in liver fat deposition observed when sucrose was replaced by cerelose or dextrin, like the improvement in growth discussed earlier, can probably be attributed to improved utilization of dietary protein.

The exception (experiment 2) in which dextrin caused no reduction in liver fat with 9% casein, although it did with 11% casein, indicates that the level of dietary casein necessary for the demonstration of this effect may be a critical factor. Threonine, which also causes a reduction in liver fat when added to the basal diet (Singal et al., '53a; Harper et al., '53a), is less effective with lower levels of dietary protein (unpublished results). Possibly the failure of Sauberlich ('53) to demonstrate an effect of the type of dietary carbohydrate on liver fat deposition may have been due to the low protein of the diet employed.

The lack of effect of the type of dietary carbohydrate on liver fat deposition when other proteins were fed may indi-

cate that a specific amino acid balance is required before such an effect can be shown. Reference has already been made to the specific effect of threonine. The higher protein content of the wheat gluten diets may account for the failure to obtain high liver fat deposition with either of these; the high nitrogen content (15.0%) and the high threonine content (7.3%) of fibrin may account for the similar results with this protein. The low nitrogen content of egg albumin (12.2%) may have contributed to the failure of dextrin to lower liver fat deposition when this protein was fed. Even the addition to the albumin diet of lysine, which causes a reduction of liver fat in rats fed lysine-deficient amino acid diets (Singal et al., '53b), was without effect.

Although the threonine content of zein is higher than that of gelatin (3.0% and 1.9%, respectively), a greater reduction in liver fat was observed in rats fed 6% gelatin than in those fed 6% zein. Since dietary threonine is very effective in reducing the liver fat of animals fed such diets, these results support the observation that the threonine in zein is not readily available (Geiger, Courtney and Geiger, '52).

The results of the 12-week experiment (table 4) indicate that the fat accumulation occurs during the period of most rapid growth. It coincides with the period of highest protein requirement and decreases as the animals mature and their amino acid requirements are reduced. Unpublished work of the authors indicates that diets of much lower protein content are required to cause high liver fat in more mature rats.

The results of the xanthine oxidase determinations indicate that when sucrose is replaced by dextrin, the protein of 9% casein diets is more efficiently utilized. Thus the sparing effect of dextrin can be shown whether xanthine oxidase, growth or liver fat deposition is used as the criterion.

SUMMARY

The influence of the type of dietary carbohydrate on growth, liver fat deposition and liver xanthine oxidase activity of rats fed *low*-protein diets has been studied.

Dextrin supported better growth of rats than sucrose, whether the protein in the diet was supplied as casein, egg albumin or wheat gluten or whether a mixture of amino acids simulating casein was used. With low-casein diets cerelose supported growth which was intermediate, while with fructose growth was slightly below that obtained with sucrose.

The liver fat deposition of rats fed low-casein diets or amino acid diets of similar composition was reduced when the sucrose in the diet was replaced by dextrin. This effect of dextrin on liver fat deposition could not be demonstrated when other proteins were fed.

Xanthine oxidase activity was higher in the livers of rats fed low-casein diets in which sucrose was replaced by dextrin.

The implications of the results have been discussed and it has been suggested that each of these observations can be explained as a result of improved utilization of dietary protein when the sucrose in low-protein diets is replaced by dextrin.

ACKNOWLEDGMENTS

We are indebted to Merck and Co., Rahway, New Jersey, for some of the crystalline vitamins; to Wilson and Co., Chicago, Ill., for gelatin; and to the Abbott Laboratories, Chicago, Ill., for the liver oil.

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THE VANDERBILT COOPERATIVE STUDY OF MATERNAL AND INFANT NUTRITION [1]

I. BACKGROUND. II. METHODS. III. DESCRIPTION OF THE SAMPLE AND DATA

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ONE FIGURE

(Received for publication August 10, 1953)

INTRODUCTION

Numerous indeed are the reports of investigations of the influence of maternal nutrition upon the course of pregnancy or the health of the infant. Valuable reviews containing extended bibliographies exist (Garry and Stiven, '36; Garry, '44; Fleming, '44; Mackay, '44; Warkany, '45; Garry and Wood, '46; Toverud, Stearns and Macy, '50; Burke and Stuart, '51).

[1] Financial assistance which has made possible this program has been generously provided by grants from the following organizations: The Nutrition Foundation, the International Health Division of The Rockefeller Foundation, the U. S. Public Health Service [RG-278 through RG-A-4(C6)], the Tennessee Department of Public Health, and Distillation Products, Inc.

The reasoning upon which such work is based may be epitomized as follows: The increased physiologic nutritional requirements during pregnancy and lactation enhance the likelihood of the development of a nutritional deficiency during the reproductive interlude. (The reproductive interlude is defined as the period during which the maternal organism provides the nutrients for the fetus and infant; i.e., from conception through parturition and lactation.) Metabolic adjustments during this period may so alter metabolism in some patients that a conditioned deficiency state may occur despite what would otherwise be an adequate dietary intake.

It has been hypothesized that a number of diseases of unexplained etiology which are peculiar to the reproductive interlude may be related to nutritional level. These include conditions such as hyperemesis gravidarum, pre-eclampsia, eclampsia, puerperal fever, excessive hemorrhage at delivery, abortion, stillbirth, premature birth, congenital malformations, hemorrhagic disease of the newborn, and the like. Thus Ebbs, Tisdall and Scott ('41) supplemented the diets of 90 women judged to have poor dietaries during pregnancy and observed, in contrast to the experience of a control group of 120 women with poor dietaries left unsupplemented, a lower incidence of miscarriages, stillbirths and prematurity, and a lower frequency of such complications during pregnancy as anemia and toxemia. Burke et al. ('43) classified 216 cases according to dietary history and concluded from their studies that there exists a significant relationship between the diet of the mother during pregnancy and the condition of the infant at birth and during the first two weeks of life. Furthermore, they observed complications in the prenatal period in 58% of the women judged to have poor dietaries and in only 32% of the women with good dietaries. On the other hand, Williams and Fralin ('42) studied the dietaries of 514 women during pregnancy but were unable to demonstrate a correlation between the course of pregnancy and the nutrient intake of the mother, and Smith ('47) reported that semistarvation

was associated with a reduced incidence of toxemia and was without effect upon the incidence of stillbirth, neonatal death or lactation.

Consideration of (1) the differences in the results of such studies as these, (2) the wide variation in the frequency of reported occurrence of the various abnormalities of pregnancy, (3) the absence of a high degree of correlation among laboratory measurements, physical signs and dietary findings, and (4) the resultant failure to define "nutriture," emphasizes the following requirements which should be met in studying the relationship among maternal nutritional level, the course of pregnancy and the health of the infant:

(a) A precise definition of the nutritional level of the subjects. This definition should be reproducible within quantitatively defined limits and not be dependent upon subjective ratings.

(b) The nutritional appraisal must take into account the changing state of the woman during the reproductive interlude. This dynamic process must be described by longitudinal methods.

(c) A clear indication of the degree to which the pregnant women studied may represent the general population of gravid women. This consideration will determine the generality of the conclusions.

The physiologic changes which are manifested in biochemical nutritional measurements during the reproductive interlude have not been adequately described, and the studies discussed above have been based primarily upon estimated dietary intakes. Accordingly, it was impossible to specify a nutritional level during pregnancy below which one might expect health impairment or above which little or no benefit could be expected to accrue.

Investigations of the problem must reckon with the existing dietary habits and nutritional levels of the women studied. Even supplementation of the diet of a group does not avoid this requirement, for it is essential that adequate characteri-

zation of prevailing nutriture precede the institution of supplementation.

It is the purpose of this study to explore methods of characterizing the nutritional state of pregnant women, to describe the nutriture of the pregnant women who entered the prenatal clinic of Vanderbilt University Hospital and to study the relationships between their nutriture and the course and outcome of pregnancy.

I. BACKGROUND

Early in 1945 six groups in the Vanderbilt University School of Medicine found that their separate interests had converged upon the subject of nutrition in pregnancy. The Departments of Preventive Medicine and of Obstetrics and Gynecology had established a system of analysis of pertinent clinical data relating to patients delivered in the hospital. The Department of Pediatrics, through its interest in problems of the neonatal period and especially of prematurity, had joined in the study organized by the other two departments and data for the newborn infants were included in the analyses. Workers in the Department of Biochemistry had long been interested in iron metabolism, its alteration during pregnancy, and its behavior in early infancy. Finally, nutrition surveys of population groups had been an area of intensive activity by several investigators in the Departments of Medicine and of Biochemistry, through the work of the Tennessee-Vanderbilt Nutrition Project. These surveys had provided a background of data on the nutritional status of population groups in Middle Tennessee (Youmans et al., '42–'43) and of similar groups in North Carolina (Darby and Milam, '45; Milam and Darby, '45). They revealed that existing diets in these areas were susceptible to improvement in the intake of several factors and, further, that in the late 1930's there had existed recognizable protein deficiency in the area around Nashville (Youmans et al., '43).

Analysis of the obstetrics data in Vanderbilt University Hospital between 1942 and 1944 had revealed a higher puer-

peral fever rate in patients admitted for delivery to the ward services than in those admitted to the private services. Furthermore, there appeared to be a negative relationship between the puerperal fever rate and the values for hemoglobin and packed cell volume at the time of admission to the hospital for delivery. Between 1941 and 1944 the incidence of toxemia was also higher for ward than for private patients. While the reasons for these differences were not known, it seemed possible that poorer diets associated with the lower economic status of the ward group might be an important factor.

In view of the many reports which emphasized the importance of nutrition in such relationships, and of the demonstrated existence of less-than-ideal dietary intakes in segments of the population in this region, we re-analyzed available unpublished data from nutrition surveys made by our group in an effort to obtain some indication of the nutriture of the pregnant women in the general population of this and similar regions. These data are presented in tables 1 to 5. The small size of the samples and the necessity of grouping together pregnant and lactating individuals in these analyses limit their inductive value. An examination of the tables, however, is of interest in that it reveals that the dietary pattern of the pregnant or lactating women was quite similar to the pattern observed in the non-pregnant, non-lactating group. There did not seem to be an increased average intake of nutrients during pregnancy which would correspond to the recommended increased nutrient allowance for this period. Furthermore, the laboratory studies revealed lower levels of hemoglobin, erythrocyte count, packed cell volume and serum protein concentration during pregnancy or lactation, and there occurred in some groups a lower concentration of ascorbic acid and vitamin A in the serum. Although the hydremia of pregnancy might account for some portion of these changes, the observations further strengthened the suspicion that the reproductive interlude is frequently a period of decreased nutriture. Therefore, attention to women going through this

TABLE 1

Comparison of nutritional assessments of pregnant or lactating women, and non-pregnant and non-lactating women (white), Wilson County, Tennessee,[1] 1939–1940–1941

ASSESSMENTS	NON-PREGNANT, NON-LACTATING 21–39 YEARS			PREGNANT OR LACTATING 21–39 YEARS		
	No. cases	Mean ± S.E.	Median	No. cases	Mean ± S.E.	Median
Dietary intake (daily)						
Calories	112	1,730 ± 50	1,700	12	1930 ± 220	1,875
Carbohydrates (gm)	112	234 ± 6	231	12	265 ± 34	233
Fats (gm)	112	66 ± 2	64	12	72 ± 10	67
Animal protein (gm)	112	27 ± 1	23	12	22 ± 4	18
Vegetable protein (gm)	112	28 ± 1	28	12	35 ± 5	32
Calcium (gm)	112	0.56 ± 0.03	0.53	12	0.65 ± 0.09	0.60
Iron (mg)	112	9.8 ± 0.3	9.4	12	10.7 ± 1.1	10.7
Thiamine (mg)	112	0.79 ± 0.03	0.76	12	0.70 ± 0.07	0.73
Riboflavin (mg)	112	1.07 ± 0.05	0.93	12	0.98 ± 0.16	0.90
Vitamin A (I.U.)	112	5,800 ± 300	4,900	12	4,400 ± 900	4,000
Vitamin C (mg)	112	71 ± 3	67	12	42 ± 10	30
Laboratory						
Hemoglobin (gm/100 ml)	104	12.63 ± 0.13	12.90	12	11.17 ± 0.37	11.17
RBC (millions/mm³)[2]	104	4.34 ± 0.04	4.35	12	3.99 ± 0.13	3.92
PCV (per cent)	105	39.4 ± 0.3	39.6	12	34.6 ± 1.4	34.0
MCV (cubic microns)	104	90.8 ± 0.9	90.0	12	87.9 ± 1.8	88.8
MCH (micromicrograms)	104	29.3 ± 0.3	29.2	12	28.3 ± 0.7	28.3
MCHC (per cent)	104	32.5 ± 0.2	32.5	12	32.5 ± 0.5	32.1
Serum protein (gm/100 ml)	106	7.02 ± 0.06	6.96	11	6.14 ± 0.28	6.12
Serum albumin (gm/100 ml)	105	4.69 ± 0.04	4.71	11	4.14 ± 0.29	4.12
Serum vitamin A (I.U./100 ml)	51	85 ± 3	80	6	44 ± 9	30
Serum carotene (μg/100 ml)	51	168 ± 8	194	6	100 ± 16	100
Serum vitamin C (mg/100 ml)	106	0.98 ± 0.04	0.97	11	0.53 ± 0.13	0.45

[1] Data sorted from unpublished records of study by Youmans, J. B., E. W. Patton and R. Kern ('42–'43).
[2] RBC = Red cell count.
PCV = Packed cell volume.
MCV = Mean corpuscular volume = 10 (PCV/RBC).
MCH = Mean corpuscular hemoglobin = 10 (Hgb/RBC).
MCHC = Mean corpuscular hemoglobin concentration = 100 (Hgb/PCV).

TABLE 2

Comparison of nutritional assessments of pregnant or lactating women, and non-pregnant and non-lactating women (colored), Wilson County, Tennessee,[1] 1939–1940–1941

ASSESSMENTS	NON-PREGNANT, NON-LACTATING 21–39 YEARS			PREGNANT OR LACTATING 21–39 YEARS		
	No. cases	Mean ± S.E.	Median	No. cases	Mean ± S.E.	Median
Dietary intake (daily)						
Calories	25	1,480 ± 70	1,520	18	1,400 ± 100	1,360
Carbohydrates (gm)	25	207 ± 14	216	18	208 ± 20	192
Fats (gm)	25	50 ± 4	55	18	46 ± 4	44
Animal protein (gm)	25	20 ± 2	18	18	17 ± 2	16
Vegetable protein (gm)	25	28 ± 2	25	18	32 ± 3	30
Calcium (gm)	25	0.58 ± 0.06	0.62	18	0.51 ± 0.05	0.47
Iron (mg)	25	9.5 ± 0.8	9.7	18	10.7 ± 1.0	10.7
Thiamine (mg)	25	0.71 ± 0.07	0.70	18	0.62 ± 0.06	0.63
Riboflavin (mg)	25	0.95 ± 0.09	0.94	18	0.89 ± 0.10	0.84
Vitamin A (I.U.)	25	5,800 ± 800	4,800	18	7,400 ± 1,100	10,000
Vitamin C (mg)	25	60 ± 9	45	18	66 ± 9	65
Laboratory						
Hemoglobin (gm/100 ml)	25	11.67 ± 0.39	11.71	15	10.72 ± 0.49	11.21
RBC (millions/mm^3)	25	4.25 ± 0.10	4.27	17	4.11 ± 0.12	4.09
PCV (per cent)	25	37.9 ± 0.7	37.7	17	35.2 ± 1.0	35.8
MCV (cubic microns)	25	87.9 ± 1.6	87.1	17	84.7 ± 3.0	83.1
MCH (micromicrograms)	25	27.4 ± 0.7	27.6	17	27.0 ± 1.2	27.2
MCHC (per cent)	25	31.2 ± 0.7	30.9	17	32.0 ± 0.7	32.2
Serum protein (gm/100 ml)	25	6.82 ± 0.11	6.78	17	6.90 ± 0.18	6.81
Serum albumin (gm/100 ml)	24	4.31 ± 0.14	4.44	16	4.30 ± 0.14	4.17
Serum vitamin C (mg/100 ml)	25	0.79 ± 0.09	0.80	17	0.67 ± 0.10	0.62

[1] Data sorted from unpublished records of study by Youmans, J. B., E. W. Patton and R. Kern ('42–'43).

TABLE 3

Comparison of nutritional assessments of pregnant, lactating, and non-pregnant, non-lactating women (white), Alamance County, N. C.,[1] July, 1943 through June, 1944

ASSESSMENTS	NON-PREGNANT 15–45 YEARS			PREGNANT 14–45 YEARS			LACTATING 15–45 YEARS		
	No. cases	Mean ± S.E.	Median	No. cases	Mean	Median	No. cases	Mean	Median
Dietary intake (daily)									
Calories	98	1,840 ± 40	1,850	4	1,750	1,750	4	2,380	2,380
Carbohydrates (gm)	98	218 ± 5	220	4	188	183	4	288	275
Fats (gm)	98	82 ± 2	84	4	85	90	4	110	110
Protein (gm)	98	58 ± 1	58	4	58	60	4	75	70
Calcium (gm)	98	0.64 ± 0.03	0.58	4	0.80	0.70	4	1.15	1.15
Iron (mg)	98	11.2 ± 0.4	11.0	4	8.5	8.7	4	14.5	13.0
Thiamine (mg)	98	1.01 ± 0.03	0.96	4	0.75	0.70	4	1.45	1.20
Riboflavin (mg)	98	1.27 ± 0.06	1.18	4	1.25	1.20	4	1.70	1.60
Nicotinic acid (mg)	71	9.0 ± 0.3	8.5	3	8.0	8.0	4	11.5	10.3
Vitamin A (I.U.)	98	4,800 ± 400	3,700	4	4,500	5,000	4	9,500	6,500
Ascorbic acid (raw foods, tomatoes or citrus fruits; mg)	98	36 ± 3	29	4	48	40	4	62	60
Laboratory									
Hemoglobin (gm/100 ml)	148	12.69 ± 0.12	12.90	6	11.00	11.33	14	12.14	12.60
RBC (mm^3)	148	4.48 ± 0.03	4.46	6	3.75	3.83	13	4.40	4.38
PCV (per cent)	148	38.5 ± 0.3	38.5	6	30.8	31.7	13	37.5	37.5
MCV (cubic microns)	148	85.9 ± 0.5	85.8	6	83.3	84.0	13	86.5	87.5
MCH (micromicrograms)	148	28.7 ± 0.3	28.6	6	27.5	27.5	13	28.3	28.5
MCHC (per cent)	148	33.2 ± 0.2	33.0	6	34.2	33.8	13	32.1	32.3
Serum protein (gm/100 ml)	80	7.40 ± 0.05	7.34	4	6.25	6.33	10	7.50	7.50
Serum albumin (gm/100 ml)	80	4.58 ± 0.03	4.61	4	3.50	3.50	10	4.70	4.62
Serum vitamin A (I.U./100 ml)	147	92 ± 2	86	6	57	55	13	103	97
Serum carotene (I.U./100 ml)	148	282 ± 11	247	6	287	309	13	264	238
Serum vitamin C (mg/100 ml)	149	0.57 ± 0.03	0.49	6	0.47	0.35	13	0.30	0.21

[1] Data sorted from unpublished records of study by Darby, W. J., and D. F. Milam ('45).

TABLE 4

Comparison of nutritional assessments of pregnant, lactating, and non-pregnant, non-lactating women (colored), Alamance County, N. C.,[1] July, 1943 through June, 1944

ASSESSMENTS	NON-PREGNANT, NON-LACTATING 15–45 YEARS			PREGNANT OR LACTATING 15–45 YEARS		
	No. cases	Mean ± S.E.	Median	No. cases	Mean	Median
Dietary intake (daily)						
Calories	26	1,620 ± 100	1,470	5	1,780	1,620
Carbohydrates (gm)	26	206 ± 14	193	5	155	162
Fats (gm)	26	67 ± 4	65	5	75	62
Protein (gm)	26	56 ± 3	52	5	59	55
Calcium (gm)	26	0.66 ± 0.05	0.63	5	0.81	0.82
Iron (mg)	26	9.8 ± 0.5	9.7	5	11.4	11.5
Thiamine (mg)	26	1.05 ± 0.08	0.97	5	1.18	1.15
Riboflavin (mg)	26	1.28 ± 0.11	1.10	5	1.54	1.30
Nicotinic acid (mg)	13	7.7 ± 0.6	8.0	2	9.0	9.0
Vitamin A (I.U.)	26	5,400 ± 800	4,200	5	9,900	10,800
Ascorbic acid (raw foods, tomatoes or citrus fruits; mg)	26	42 ± 6	29	5	49	48
Laboratory						
Hemoglobin (gm/100 ml)	39	12.32 ± 0.19	12.47	9	10.18	10.50
RBC (mm^3)	39	4.48 ± 0.07	4.54	9	4.36	4.25
PCV (per cent)	39	37.9 ± 0.5	38.2	9	32.9	33.0
MCV (cubic microns)	39	86.0 ± 1.0	86.2	9	78.3	81.0
MCH (micromicrograms)	39	27.9 ± 0.4	28.3	9	23.7	23.9
MCHC (per cent)	39	32.5 ± 0.2	32.8	9	31.0	30.3
Serum protein (gm/100 ml)	27	7.40 ± 0.12	7.36	4	6.88	6.85
Serum albumin (gm/100 ml)	27	4.46 ± 0.06	4.46	4	3.60	3.60
Serum vitamin A (I.U./100 ml)	40	85 ± 4	87	9	81	82
Serum carotene (I.U./100 ml)	40	305 ± 25	280	9	253	238
Serum vitamin C (mg/100 ml)	40	0.68 ± 0.07	0.65	9	0.49	0.35

[1] Data sorted from unpublished records of study by Darby, W. J., and D. F. Milam ('45).

TABLE 5

Comparison of lactating and non-lactating white females
Williamson County, Tenn.,[1] January–June, 1944

ASSESSMENTS	NON-LACTATING			LACTATING		
	No. cases	Mean ± S.E.	Median	No. cases	Mean ± S.E.	Median
Dietary intake (daily)						
Calories	31	1,970 ± 130	1,840	11	2,350 ± 210	2,220
Carbohydrates (gm)	31	228 ± 13	218	11	295 ± 24	300
Fats (gm)	31	92 ± 7	86	11	98 ± 11	92
Animal protein (gm)	31	40 ± 3	38	11	37 ± 8	32
Total protein (gm)	31	66 ± 4	61	11	72 ± 10	72
Calcium (gm)	31	0.88 ± 0.08	0.80	11	0.91 ± 0.13	0.92
Iron (mg)	31	10.7 ± 0.6	10.3	11	12.9 ± 1.6	13.8
Thiamine (mg)	31	1.33 ± 0.09	1.28	11	1.32 ± 0.28	1.02
Riboflavin (mg)	31	2.00 ± 0.15	1.88	11	1.79 ± 0.28	1.50
Nicotinic acid (mg)	31	8.5 ± 0.6	8.0	11	9.5 ± 1.4	8.0
Vitamin A (I.U.)	31	5,500 ± 600	5,200	11	5,100 ± 900	5,800
Vitamin C (mg)	31	38 ± 3	39	11	38 ± 5	39
Laboratory						
Hemoglobin (gm/100 ml)	30	12.85 ± 0.14	13.08	11	12.19 ± 0.28	12.50
PCV (per cent)	30	40.2 ± 0.4	40.5	11	38.5 ± 0.8	39.6
Serum albumin (gm/100 ml)	31	4.87 ± 0.05	4.89	11	4.95 ± 0.07	4.90
Serum vitamin A (I.U./100 ml)	31	104 ± 4	102	10	116 ± 10	115
Serum carotene (μg/100 ml)	31	110 ± 8	109	11	89 ± 14	80
Serum vitamin C (mg/100 ml)	31	0.35 ± 0.06	0.27	11	0.13 ± 0.05	0.07
Weight						
$\frac{\text{Actual weight}}{\text{Standard weight}} \times 100$	32	104 ± 4	102	11	96 ± 4	94

[1] Robinson, W. D., R. Steinkamp and M. Kaser 1944 A study of the nutritional status of a population living in the First District of Williamson County, Tennessee. Unpublished manuscript.

interlude should reveal individuals suffering from the effects of nutritional deficiencies which might not be recognizable without the added stress of reproduction.

In order to investigate the problems suggested by the above background material, the interested members of the several departments concerned organized a Study of Maternal and Infant Nutrition. The design of this study was longitudinal and included well-considered dietary, biochemical and clinical technics for the estimation of nutritional status. The sample was unselected — it constituted all of the pregnant white women who presented themselves for prenatal care at the Vanderbilt University Hospital Out-Patient Clinic during the period of the study.

The findings, therefore, will allow an objective quantitative description of changes in nutritional physiology which occur in pregnancy and post partum. Furthermore, they provide the first extensive quantitative assessment of nutritional level which may be related to the health performance during pregnancy of all persons who seek care at a prenatal center such as ours. Thus the study allows for a more critical exploration than has previously been possible of the role of nutrition as a determining factor in the healthfulness of pregnancy.

Since pregnancy represents a stress period, the findings of the investigation have implications with respect to the adequacy of the nutritional level of a considerable segment of the population at large of the region. Finally, the careful analysis of the study has brought to light many important concepts in the methodology of such surveys.

II. METHODS

On the first prenatal visit of each white woman attending the Obstetric Clinic, in addition to the usual medical and obstetrical history and examination, a special nutritional physical examination was made, and a sample of venous blood was taken for estimation of hemoglobin content; erythrocyte count; packed cell volume; serum ascorbic acid, caro-

Special Study of Nutriture in Pregnancy
Physical Examination

Date _____ Name _____
Last name First name

Expected
Delivery Date _____ History Number _____ Age _____

Trimester of Exam _____ Examiner _____

Weight _____ lbs. (to next lowest)

Height _____ inches (to next lowest)

General Nutriture:
- Under
- Satisfactory
- Obese

Skin:
- Dry
- Keratosis pilaris
- Icthyosis
- Hyperkeratotic lesions of extensor surfaces
- Seborrhea of face
- Atrophy
- Symmetrical dermatitis of exposed surfaces

Eyes:
- Photophobia
- Excessive lacrimation
- Conjunctival injection
- Pingueculae
- Conjunctival dryness
- True Bitot's spots

Nose:
- Plugs at nasolabial area

Mouth:
- Angular fissures
- Angular scars
- Cheilosis

Teeth:
- Adentia
- Plates

Gums:
- Red
- Swollen
- Pyorrhea

Tongue:
- Pale
- Increased redness
- Fissuring
- Papillary atrophy
- Patchy denuded areas
- Hypertrophic fungiform papillae

Lower Extremities:
- Edema
- Varicose veins

Neurological:
- Loss of knee jerk
- Loss of ankle jerk
- Loss of vibratory sense over toes
- Calf tenderness

REMARKS:
- Positive history of night blindness

DIRECTIONS:
- If organ examined is "Normal" indicate by a ( ) after heading; if present, circle the condition. Do not leave any blanks.
- Use space "REMARKS" for descriptions where desired.
- Record height and weight to next lower inch or pound, thus 62½ inches is recorded as 62 inches, and 130¾ lbs. is recorded as 130 lbs.

Figure 1

tene and vitamin A; total serum proteins; and serum albumin. No prescriptions of dietary supplements were given on this occasion.

Nutritional physical examinations (fig. 1) were made by as few observers as was feasible under the working conditions of the clinic. Most of these were carried out by physicians primarily concerned with the study of nutrition in pregnancy or by members of the house staff of the rank of Assistant Resident or Resident. In all instances, the examiner's initial observations were made under the direction of a member of the Division of Nutrition after an orienting discussion and a demonstration of the signs which were to be observed. In addition, duplicate examinations were occasionally made by two or more observers. It was hoped thereby to standardize the observations on physical findings in order that they might be strictly comparable.

On the second prenatal visit (usually one week following the first), urinary load tests for thiamine, riboflavin and N′-methylnicotinamide were run. These consisted of the oral administration [2] of a standard dose of 5 mg of thiamine hydrochloride, 5 mg of riboflavin, and 50 mg of nicotinamide. Simultaneously, 200 ml of tap water were given. The urine voided during the next two hours was collected, the volume made up to 200 ml with distilled water, 2 ml of glacial acetic acid added, and the specimen stored in a brown bottle in a refrigerator for a period not exceeding one week before analysis.

On this second visit, a nutritionist provided the patient with a form for the recording of all food and drink (except water) which the patient consumed during the subsequent 7 days. Space was provided for listing the kind of food and the amount in household measures consumed at each meal, between meals, and between supper and bedtime. The patient was instructed to record these data immediately after each meal and directions were given concerning the identification

[2] These vitamin preparations were kindly supplied by Dr. J. F. Biehm, Director, Abbott Research Laboratories, North Chicago, Illinois.

of foodstuffs and the estimation of quantities. No instructions for the alteration of dietary practices were included, and it was emphasized that the record was an effort to determine what the patient usually ate and not what she thought she should eat. On the third visit, which was one to three weeks later, the nutritionist discussed the completed record in detail and made notations as to the composition of mixed dishes, the size of measures, and any other pertinent information. On the basis of this 7-day food intake record, estimates were made of the average daily nutrient intakes, using the short method of calculating the nutritive contents of diets as studied and adopted for Middle Tennessee by Steinkamp, Robinson and Kaser ('45). The validity of this procedure was discussed in the original description of the method and has been further examined by Kaser, Steinkamp, Robinson, Patton and Youmans ('47).

During a portion of the study, a sample of blood was taken from the patients on their third visit and analyzed for total tocopherol content. During the earlier period of the investigation, patients received tracer doses of radioactive iron in order to study the absorption of this element; the methods employed and the findings of this portion of the study have been published by Hahn and co-workers ('51).

The *procedures* used for the laboratory measurements are listed below:

Hemoglobin: The absorption by laked diluted blood at 540 mμ was measured in an Evelyn photoelectric colorimeter immediately after the addition of a drop of ammonium hydroxide (Rubicon Co.). The method was calibrated against measurements of the oxygen capacity by the Van Slyke procedure.

Erythrocyte count: Pipettes and counting chambers were certified by the U. S. Bureau of Standards. Oxalated or heparinized venous blood was employed. A single pipette filling was made; the diluent was Hayem's solution (Todd and Sanford, '43). Both sides of a double chamber were filled and 160 squares enumerated.

Packed cell volume: Wintrobe's ('33) macro procedure was followed.

Total serum proteins: The specific gravity method of Linderstrom-Lang as described by Lowry and Hunter ('45) was employed.

Serum albumin: Kingsley's ('40) method of separation of albumin and globulin was followed by a Kjeldahl digestion and titration, as described by Ma and Zuazaga ('42).

Serum vitamin A and serum carotene: The macro adaptation of the Carr-Price reaction, as described by Kaser and Stekol ('43), was used.

Serum ascorbic acid: The photoelectric procedure using 2,6 dichlorophenol-indophenol, as outlined by Bessey ('38), was employed.

Plasma tocopherol: Total tocopherols were estimated by the method of Quaife and Harris ('44) as modified by Quaife and Biehler ('45).

Thiamine: Urinary thiamine was determined by the thiochrome procedure as described by Perlzweig, Kamin, Gue and Blalock ('45).

Riboflavin: Urinary riboflavin was measured by the direct method of Ferrebee ('40).

N'-methylnicotinamide: Urinary N'-methylnicotinamide was determined by the acetone method described by Huff, Perlzweig and Tilden ('45).

Except for the laboratory estimations of hemoglobin concentration, erythrocyte counts and packed cell volume, none of the above data became a part of the permanent clinic record of the patient. Thereby the observations on nutritional level could not influence the subsequent handling of the particular patient, since the obstetrician remained ignorant of the nutritional findings. The nutritionist for the study gave no dietary advice to the patient during her pregnancy. (The obstetrician gave the usual basic advice on the patient's second visit about the drinking of milk, eating of foods which are good sources of iron, and so forth. Supplementation was not routine and no dietary supplements were ever given until after the second prenatal visit. The details of prescriptions for dietary supplements and the effects will be discussed in a later paper. Obese patients were told to reduce intakes of starches and sweets. Only extremely obese patients and cases such as diabetics were sent to the hospital clinic dietitian for special diets.)

Once during each subsequent trimester of pregnancy the dietary studies, physical examination and laboratory tests (except for plasma tocopherol and radioactive iron uptake) were repeated. The physical examination and the laboratory observations were repeated again at the time of the 6-week post-partum examination. It was not feasible to obtain an additional dietary record during the post-partum period.

Heparinized samples of venous blood from the newborn were obtained during the third to 6th day of life, and estimations were made of hemoglobin content, erythrocyte count, packed cell volume and plasma proteins. This study was repeated on blood samples drawn from the babies on their subsequent visits to the Well-Baby Clinic. In most instances but one follow-up record was possible, this most often during the second month of life. During the latter part of the study special efforts were made to obtain additional samples from babies of mothers whose third-trimester hemoglobin levels were at the extremes of the range, in order to study whether a relationship existed between the hemoglobin concentrations in the blood of mothers and infants.

From the hospital pregnancy and delivery records and the pediatric record, information concerning the clinical course of the mother and child was extracted. This included a great variety of data, such as the clinical history of previous pregnancies, prenatal history during the present pregnancy, existence of complications (toxemia, perverted pregnancy and the like), type and duration of labor, weight of the placenta, existence of puerperal morbidity or fever, birth weight, birth length, presence of malformations and maximum weight loss of the infant. To avoid bias this material was taken from the record by a person uninformed as to the results of the nutritional appraisals.

Throughout this study, the following *classifications* were adhered to:

Pre-eclampsia: Diagnosis is made with the appearance of any two of the following, not previously existing: (1) a systolic blood pressure of 140 mm of mercury or more for two days or longer, (2) edema of no obvious etiology involving at least the ankles and legs, (3) proteinuria which is present for two days or more.

Eclampsia: The symptoms of pre-eclampsia, plus coma or convulsions.

Toxemia: Diagnosis of pre-eclampsia, eclampsia or hyperemesis gravidarum.

Puerperal fever or morbidity: An oral temperature of 100.4° for two successive days, excluding the first 24 hours post partum, during the hospital stay of the patient. Temperature recorded 4 times daily.

Puerperal complications: Complications of the puerperium involving the reproductive tract, breast, genitourinary, gastrointestinal, or upper respiratory tract, or thrombophlebitis, or anemia, with or without fever.

Abortion: A fetus less than 28 cm long, less than 400 gm in weight and born prior to 20 weeks of gestation (missed abortions are exeluded).

Stillbirth: A baby born dead, over 20 cm long, over 400 gm in weight, and born after 20 weeks of gestation.

Premature: An infant weighing 400 to 2,500 gm at birth.

Infant death: Deaths among Vanderbilt Hospital deliveries include those to time of discharge. Deaths among deliveries elsewhere are those within 28 days among cases where outcome of pregnancy was reported to us.

Congenital anomaly: Malformations noted either clinically or at autopsy during the first two weeks after delivery, including minor anomalies of the skin and appendages of cosmetic importance, and defects which in the opinion of the pediatric staff constituted an actual or potential threat to healthy development.

Weeks of gestation: Computed from the date of the beginning of the last normal menses, except in a few cases where this resulted in in a value of more than 44 weeks of gestation at delivery. In these cases, the number of weeks of gestation at delivery was arbitrarily estimated as 40.

Trimester of pregnancy: The first 13 weeks of gestation are referred to as the first trimester, 14 through 26 weeks as the second trimester, 27 weeks until delivery as the third trimester.

Upon completion of the study, all data pertaining to the nutritional and health assessments of both mothers and infants were coded and punched onto International Business Machine punch cards. Seven basic punch cards were required to contain the data. These basic cards served as the sources of initial data on distributions and, also, as a basis for the punching of secondary correlation cards, of which a total of 64 decks was prepared and studied.

III. DESCRIPTION OF THE SAMPLE AND DATA

Each white woman who presented herself to the Obstetric Clinic of the Vanderbilt University Hospital for prenatal care during the period between September, 1945 and February, 1949, was entered in the study. A total of 2,338 pregnancies was studied. This number includes two or more admissions for 260 women, 248 of whom were observed during

two, 11 during three, and one during 4 pregnancies. The inclusion of new cases was terminated in February of 1949, since the initial goal of 2,000 cases had been exceeded and it was judged that the number was sufficient for interpretation of the findings.

The patients were from families of low to moderate income status. Admission to the clinic, within the restrictions of minimum to maximum gross annual incomes of $1,200 to $3,000, was determined by the hospital Social Service on the basis of needs, debts and dependency.[3]

The outcome of pregnancy is unknown in 209 cases. In an additional 83 cases, the outcome (whether a live birth, stillbirth, abortion or infant death) is known, but delivery did not occur in our hospital and the usual records for the mother and newborn infant were not obtained.[4] Thus, this study includes 2,046 consecutive prenatal cases which were cared for and delivered in the Vanderbilt University Hospital. In the analyses to follow, the 209 cases with unknown outcome are omitted entirely, and the 83 cases of known outcome which were not delivered in this hospital are included in the analyses

[3] When this study was initiated in 1945, some patients were admitted for out- and in-patient care without charge; others were charged at minimum rates. In 1947 the pay system was altered so that all patients were charged minimum fees. Fees per visit to the out-patient clinic ranged from $0.25 in 1945 to $1.00 in 1949, and minimum total charges for hospitalization for delivery ranged from $21 to $60 over this interval.

[4] The places of delivery for the 83 patients with known outcomes but who were not delivered in Vanderbilt Hospital were as follows:

34 — Home delivery service, Vanderbilt (discontinued in 1947)
4 — Home delivery service, not Vanderbilt
15 — Deliveries outside the city
7 — Deliveries in other hospitals in Nashville area
3 — Deliveries en route to hospital or in emergency room
20 — Abortions (see below)
83 — Total

Of the 20 abortions, 18 presumably were unattended at home, one occurred in another hospital, and one was attended by a private physician. They represent half of the 40 known abortions which occurred in the total study group. There were three stillbirths and three infant deaths in this group. These are somewhat more than would be expected, according to the rates for the other patients. The higher frequencies are not statistically significant when considered separately, but that of the combined groups is significant at the probability level of 0.03. The higher incidence of abortions outside of the hospital deliveries is to be expected from the nature of the disorder.

of data on laboratory, dietary and physical examination findings during pregnancy and the post-partum period and in the studies of the relation of these findings to the outcome of pregnancy. This group of 83 is of necessity omitted from studies involving information from the hospital records at delivery of the newborn. Not included in the study group are 66 women tentatively admitted to the prenatal clinic but later declared to be non-pregnant, some of whom might have had early abortions.[5]

A study was made of all available information on the 209 excluded cases to determine, insofar as possible, the character of the group and the effect of such exclusion on the results. Analysis of the laboratory, dietary and physical examination data for these cases with unknown outcome did not reveal significant differences between them and the patients who continued in the study (see table 6). The nature of the reasons for not continuing in the study,[6] when given, and the agreement between the data obtained for the groups with known and unknown outcomes, lead us to conclude that this excluded group was not predictably different from the group who remained, and that failure to follow all patients through delivery did not result in an important bias in our results.

The basic group of cases, summarized in table 7, consisted of 2,129 women, 2,046 of whom were delivered in Vanderbilt University Hospital. The total number of live-born infants was 2,079, 2,018 of whom were born in our hospital. There were no maternal deaths. There were 40 abortions, 33 stillbirths and 45 infant deaths. Of the 23 sets of twins, all but one set were delivered at Vanderbilt University Hospital.

[5] Since time of entry in relation to stage of gestation was dependent upon when the woman decided to present herself at the clinic, our data on abortions should not be construed as indicating the frequency of abortions generally.

[6] The reason for failure to return to the Vanderbilt prenatal clinic or hospital was obtained for 126 of the 209 cases with unknown outcome:

53 — moved away
50 — transferred to a private physician
18 — transferred to another hospital clinic
2 — venereal disease, refused treatment
2 — uncooperative psychoneurotic patients
1 — transferred to a county tuberculosis hospital

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TABLE 6

Comparison of mean ± S. E. of selected nutrient intake and blood levels, women followed through delivery versus women lost from study before delivery. (Items shown are representative of lack of significant differences in other measurements)

STAGE OF GESTATION	DAILY CALORIE INTAKE Outcome of pregnancy		DAILY PROTEIN INTAKE (GM) Outcome of pregnancy		SERUM VITAMIN A (I.U./100 ML) Outcome of pregnancy	
	Known	Unknown	Known	Unknown	Known	Unknown
1st trimester	2,140 ± 40	2,020 ± 110	75.0 ± 1.3	74.6 ± 4.3	111 ± 2	118 ± 4
2nd trimester	2,200 ± 20	2,110 ± 60	75.3 ± 0.6	73.8 ± 2.4	112 ± 1	117 ± 2
3rd trimester	2,020 ± 20	1,930 ± 100	69.8 ± 0.5	71.9 ± 3.6	100 ± 1	99 ± 3

STAGE OF GESTATION	TOTAL SERUM PROTEIN (GM/100 ML) Outcome of pregnancy		HEMOGLOBIN (GM/100 ML) Outcome of pregnancy		RED BLOOD COUNT (MILLIONS/MM^3) Outcome of pregnancy	
	Known	Unknown	Known	Unknown	Known	Unknown
1st trimester	6.89 ± 0.02	6.86 ± 0.06	13.02 ± 0.07	12.78 ± 0.14	4.33 ± 0.02	4.31 ± 0.06
2nd trimester	6.54 ± 0.01	6.58 ± 0.04	12.22 ± 0.03	12.25 ± 0.10	4.09 ± 0.01	4.13 ± 0.04
3rd trimester	6.34 ± 0.01	6.38 ± 0.04	11.66 ± 0.03	11.67 ± 0.14	3.97 ± 0.01	3.95 ± 0.05

The distribution of the women according to age and parity is given in table 8. Table 9 gives the incidence of certain conditions of special interest within the group delivered in our hospital.

The stage of pregnancy at the time of the first clinic visit varied between 5 to 41 weeks of gestation. There were 344 women who entered the study during the first trimester of pregnancy; 1,086, the largest group, entered during the second trimester; and 699 during the third trimester. Some interesting differences in the laboratory, dietary and physical

TABLE 7

Outcome of pregnancy for total study group

OUTCOME	DELIVERED AT V.U.H.	DELIVERED ELSEWHERE	TOTAL
Total pregnancies	2,046	83	2,129 [2]
Abortions	20	20	40
Stillbirths	30	3	33
Livebirth confinements	1,996	60	2,056
Twin sets [1]	22	1	23
Infants delivered alive	2,018	61	2,079
Infant deaths [1]	42	3	45
Infants alive at discharge	1,976	58	2,034

[1] Twins were all liveborn; 42 infant deaths in V.U.H. included 5 twins in 4 sets.
[2] Total with known outcome (2,129) = prenatal clinic entries (2,338) — unknown outcomes (209).

examination data among patients who attended the clinic first during different periods of pregnancy will be presented in a later paper.

At least one laboratory record was obtained for each patient, at least one physical examination on all but 9 of the entrants, and one or more diet records on all but 282 women. The completeness of the repeated observations obtained for our patients is indicated in table 10. The failure of the women to keep the dietary record was somewhat more common than was failure to attend the clinic where the laboratory studies and physical examinations were done. Blood samples were drawn during the hospital stay from 1,281 of the 2,018

infants live-born in the hospital, and from 963 infants on return to the Well-Baby Clinic.

It was thought that the group who returned for the post-partum checkup might differ in some important respects from

TABLE 8

Distribution by age and parity of 2,046 cases delivered in Vanderbilt University Hospital

AGE	PARITY							TOTAL	PER CENT OF TOTAL CASES
	0	1	2	3	4	5–9	10 AND OVER		
years									
10–14	5	1						6	0.3
15–19	365	108	17					490	24.0
20–24	217	272	126	59	23	10		707	34.6
25–29	44	97	101	56	52	63	1	414	20.2
30–34	18	23	25	39	31	83	5	224	10.9
35–39	6	10	18	13	18	73	20	158	7.7
40 and over	3		3	2	2	22	15	47	2.3
Total	658	511	290	169	126	251	41	2046	100.0
Per cent of total cases	32.2	25.0	14.2	8.3	6.2	12.3	2.0	100.2	

TABLE 9

Incidence of certain special conditions among 2,046 deliveries in Vanderbilt University Hospital

CONDITION	NO. OF CASES	PER CENT OF WOMEN HAVING CONDITION
Eclampsia	10	0.5
Pre-eclampsia	93	4.5
Hyperemesis	15	0.7
Puerperal fever	110	5.4
Premature liveborn infant	115 [1]	5.6
Congenitally malformed infant	55	2.7

[1] The 115 cases involved 123 infants, 8 sets of twins, both premature, being counted as one case each. (Seven other twins were premature.)

the group who did not return. Consequently, the two groups were compared on the bases of laboratory and dietary data during pregnancy, outcome of pregnancy, and obstetrical data. No important differences were found.

It is our belief, therefore, that the cases included in this analysis are free of recognizable selection insofar as the

population of our hospital clinic is concerned. They are, of course, selected by those undetermined forces which decide that patients attend this hospital clinic. No patients have been excluded by us from the study except for the reasons stated above. Our conclusions, therefore, should be valid for guiding the program of this and similar clinics.

TABLE 10

Number of records obtained in each trimester and post partum
(2,129 pregnancies with known outcome)

TYPE OF RECORD	TRIMESTER			POST PARTUM
	I	II	III	
Laboratory	344	1,377	1,978	1,565
Physical examination	340	1,372	1,969	1,547
Dietary	278	1,222	1,665	....
Number of women in study	344	1,430	2,129	2,129

SUMMARY

The background, design, methodology and population sample of a study of nutrition in pregnancy are described. This study embraced 2,338 pregnant white women cared for in the prenatal clinic of Vanderbilt University Hospital during the period from September, 1945, to February, 1949.

The limited available data on nutritional assessments of pregnant or lactating women within the general population of the region are summarized.

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THE VANDERBILT COOPERATIVE STUDY OF MATERNAL AND INFANT NUTRITION [1]

IV. DIETARY, LABORATORY AND PHYSICAL FINDINGS IN 2,129 DELIVERED PREGNANCIES

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FIVE FIGURES

(Received for publication August 10, 1953)

INTRODUCTION

The purpose and plan of the Vanderbilt Cooperative Study of Maternal and Infant Nutrition have been presented in a preceding paper (Darby et al., '53). This, the second paper in the series, describes the nutritional characteristics of the study group of 2,129 cases [2] as indicated by records of their dietary intake, laboratory assessments and physical examinations. It will thus furnish a basis for comparison with other

[1] Financial assistance which has made possible this program has been generously provided by grants from the following organizations: the Nutrition Foundation, the International Health Division of The Rockefeller Foundation, the U. S. Public Health Service [RG-278 through RG-A-4(C6)], the Tennessee Department of Public Health, and Distillation Products, Inc.

[2] A total of 2,338 cases was admitted to the Vanderbilt prenatal clinic during the period of the study; 2,046 of these were delivered in Vanderbilt Hospital and 83 were delivered elsewhere. Hence, the group of 2,129 cases dealt with here comprise all of those for whom the outcome of pregnancy is known.

studies and also lay the groundwork for the papers to follow, which will discuss the relation among available measures of nutriture and the course and outcome of pregnancy and the attributes of the fetus.

Any evaluation of nutritional status must recognize the normal changes which are characteristic of the changing physiological state of pregnancy and the puerperium. For example, the expectation of alterations in nutrient requirements is reflected in estimates of nutrient allowances such as the Calorie Allowance of FAO (Committee on Calorie Requirements, '50), the Recommended Dietary Allowances of the Food and Nutrition Board ('48), or the Standards of the Canadian Council on Nutrition ('50). The actual intake levels of a large unselected group of pregnant women should aid in the interpretation and evaluation of such estimates.

Among the examples of biochemical alterations which are recognized as characteristic of pregnancy are hemodilution and water retention (Dieckmann and Wegner, '34; Caton et al., '51), increased urinary excretion of N′-methylnicotinamide (Darby, Cannon and Kaser, '48; Frazier, Porter and Humphrey, '49), increases in the serum concentration of carotene, tocopherol and cholesterol (Darby, Cannon and Kaser, '48), and changes in concentration of serum protein (Rinehart, '45; Coryell et al., '50; Wiehl, '50). Hitherto these alterations have not been related adequately to other measures of nutriture during pregnancy, so as to permit proper use of them as measures of nutritional status.

DATA

Dietary intakes

Table 1 summarizes the nutrient intakes as calculated from the dietary records [3] which each patient was requested to keep for a week once during each trimester of pregnancy. The

[3] Approximately 94% of the records were 7-day. The remainder were 4-, 5- or 6-day records. In all instances, average daily intakes were calculated upon the basis of actual days for which the record was obtained.

TABLE 1

Summary of average daily nutrient intakes for each trimester

NUMBER OF DIET RECORDS	FIRST TRIMESTER 278		SECOND TRIMESTER 1,222		THIRD TRIMESTER 1,665	
Nutrient	Mean	S. D.	Mean	S. D.	Mean	S. D.
Total calories	2,140	630	2,200	630	2,020	640
% of calories from carbohydrates	46	5	47	5	47	6
% of calories from fat	40	5	40	5	39	5
% of calories from protein	14	2	14	2	14	2
Protein (gm)	75	22	75	21	70	21
% animal protein	66	9	65	10	65	10
% vegetable protein	34	9	35	10	35	10
Calcium (gm)	1.1	0.4	1.1	0.4	1.0	0.4
Phosphorus (gm)	1.4	0.4	1.4	0.4	1.3	0.4
Iron (mg)	13	4	14	4	13	4
Thiamine (mg)	1.5	0.5	1.5	0.5	1.4	0.5
Niacin (mg)	12	4	12	4	11	4
Riboflavin (mg)	2.5	0.9	2.5	0.9	2.3	0.9
	P_{10}	Median	P_{90}	P_{10}	Median	P_{90}	P_{10}	Median	P_{90}
Total vitamin A activity (I.U.)	2,700	6,500	13,600	2,800	6,600	13,800	2,400	5,900	13,400
Animal vitamin A activity (I.U.)	800	2,000	5,900	900	2,100	6,100	700	1,800	5,600
Vegetable vitamin A activity (I.U.)	1,200	3,800	10,100	1,200	3,800	9,600	1,000	3,400	8,900
Vitamin C (mg)	26	66	123	25	62	120	19	55	114
Vitamin D (I.U.)	30	64	100	32	67	107	27	62	101
Thiamine/NFC (μg/calorie)	0.8	1.1	1.6	0.8	1.1	1.6	0.8	1.1	1.6

distributions were sufficiently close to the normal curve to be described adequately by the mean and standard deviation except in the instances of vitamins A, C and D and of the thiamine/non-fat calorie ratio. These latter were skewed, and are summarized by the median and the 10th and 90th percentiles. The data have been examined in relation to a number of concomitant variables such as age, parity, weight, trimester of entry of the mother into the clinic, and the season of the year when the observations were made. The detailed consideration of these variables will, for the most part, be postponed until later papers, but their effect will be considered in the interpretation of the basic findings.

It is immediately apparent that, although the average nutrient intakes were somewhat below the standards widely accepted for good nutrition, they were not sufficiently low to cause concern. The relatively wide spread of the distribution indicates that patients at different levels of intake were available for study.

Of particular interest is the change in the estimated caloric intake as pregnancy progressed. Caloric consumption not only failed to increase between the second and third trimesters but, instead, decreased by roughly 200 calories. This decreased food consumption was reflected in all of the nutrients to about the same extent, as may be judged from the constancy of the percentage contribution of the major foodstuffs to the caloric intake and from the parallelism of the changes between calories and other nutrients.

An unexpected association is that between the time in pregnancy at which the woman first attended the prenatal clinic and her nutrient intake. From table 2 it is apparent that the women tended, at their trimester of entry, to record an intake some 200 calories higher than did those women at the same period in pregnancy who had entered the clinic at an earlier trimester. Differences in the same direction as those for caloric consumption were noted for other nutrients (protein, calcium, phosphorus, iron and vitamins A, B and C), although the percentage differences varied somewhat for the different

nutrients. For example, the group entering in the third trimester had higher average values for all the nutrients than those who had entered the clinic earlier, but this difference was greater for calories than for protein, calcium, iron and ascorbic acid. It is not possible to say whether these differences are a reflection of appetite or whether perhaps they are

TABLE 2

Daily calorie intake, by trimester that women entered study, for each trimester

	MEAN CALORIE INTAKE ± STANDARD ERROR OF MEAN			NUMBER OF CASES		
	1st trim. diets	2nd trim. diets	3rd trim. diets	1st trim. diets	2nd trim. diets	3rd trim. diets
Entered 1st trimester	2,130 ± 35	2,090 ± 34	1,930 ± 35	275	279	260
Entered 2nd trimester	..	2,210 ± 18	1,960 ± 20	...	942	881
Entered 3rd trimester	..	..	2,140 ± 26	...	...	524

TABLE 3

Daily calorie intake, by season, for each trimester

(Average ± S. E.)

STAGE OF GESTATION	SEASON OF DIET RECORD				TOTAL
	Winter	Spring	Summer	Fall	
1st Trimester	2,128 ± 66	2,160 ± 76	1,998 ± 83	2,232 ± 75	2,142 ± 38
2nd Trimester	2,279 ± 37	2,191 ± 36	2,098 ± 34	2,202 ± 35	2,199 ± 18
3rd Trimester	2,105 ± 32	1,959 ± 29	1,932 ± 33	2,068 ± 32	2,020 ± 16

the result of advice given by the physicians in the clinic concerning the restriction of food to avoid excessive weight gain. Nevertheless, tables 1 and 2 clearly indicate that throughout our study the average number of calories consumed decreased during pregnancy.

Seasonal[4] trends in the mean caloric intake are shown in table 3. They were in the expected direction of higher intakes

[4] The "seasons" were defined as follows: winter, January 1 through March 31; spring, April 1 through June 30; summer, July 1 through September 30; fall, October 1 through December 31.

in fall and winter than in spring and summer. Seasonal variation in the intake of protein, calcium, phosphorus, iron and B vitamins resembled, in general, the trend for total calories, except that thiamine intake was relatively low during the summer.

Less regular variation appeared in the seasonal intake of vitamins A and C. The highest recorded intakes of vitamin A activity from vegetable sources occurred during the two periods of February through May and August through November. Second trimester diet records showed highest intakes of vitamin A from animal sources during the periods March through May and September through November, but there was little or no seasonal variation in animal vitamin A intakes in the third trimester records. During the third trimester vitamin C intakes were lower from June through October than they were during the rest of the year. However, during the second trimester ascorbic acid intake was lowest during January, June, July and September. These variations are cited to indicate that no simple generalization may be stated.

The influence of season upon the intake of nutrients will be considered in more detail in later reports. Suffice it to say that the continuous schedule of acceptance of patients into the study was effective in minimizing the seasonal effect on the various groups.

The data were examined to determine whether there were any long-term trends in the intakes of various nutrients from the early to the late period of the investigation. No trends of significant magnitude were detected. The maximum changes were an average increase of about 0.5 mg in iron and of about 0.7 mg in niacin.

The foods which supplied these nutrients are indicated in table 4, in which the food consumption per patient is expressed as the number of average servings per week. An "average serving" is that defined by Steinkamp, Robinson and Kaser ('45). The table shows the 10th, 25th, 50th, 75th and 90th percentiles of the distributions.

TABLE 4

Third trimester food intakes of 1,665 women

(Values in terms of the number of servings per week)

FOODS	% EATING NONE OF GROUP	% EATING NONE	PERCENTILES				
			10th	25th	Median	75th	90th
Biscuits		12.6	0.0	4.1	11.4	21.8	36.1
Cornbread		10.6	0.0	2.6	7.3	13.2	20.9
Cereals		1.4	5.7	10.5	16.5	23.1	29.9
	0.1						
Potatoes		5.9	1.0	3.1	5.6	8.5	11.6
	5.9						
Beef, lamb, poultry, etc.		13.0	0.0	0.8	2.5	3.9	5.7
Pork		23.4	0.0	0.6	2.5	4.5	7.0
Lunch and organ meats		40.8	0.0	0.0	1.0	3.4	5.6
Fish and seafoods		65.2	0.0	0.0	0.0	1.5	3.0
	1.3						
Legumes		13.3	0.0	1.3	3.5	6.2	9.6
	13.3						
Eggs		4.4	2.4	4.5	6.6	8.6	11.5
	4.4						
Milk		1.2	4.1	8.6	14.4	20.7	27.4
Cheese		54.6	0.0	0.0	0.0	1.7	3.5
Ice cream		43.2	0.0	0.0	0.5	2.7	5.1
	0.7						
Butter		12.5	0.0	0.8	2.5	4.7	7.8
Fats		1.3	4.0	7.2	11.8	17.6	23.9
	0.5						
Green leafy vegetables		46.9	0.0	0.0	0.8	2.9	4.8
Green and yellow vegetables		6.6	0.5	2.3	4.1	6.7	9.5
Other vegetables		4.4	1.0	2.8	4.9	7.6	10.4
	0.9						
Tomatoes		17.0	0.0	0.9	3.5	6.4	9.8
Citrus fruits		32.0	0.0	0.0	3.9	6.0	10.5
Yellow fruits		58.5	0.0	0.0	0.0	1.9	4.5
Fresh and canned fruits		18.2	0.0	0.7	3.4	6.8	10.3
Dried fruits		85.3	0.0	0.0	0.0	0.0	1.3
	2.1						
Pie		44.6	0.0	0.0	1.0	2.4	4.1
Cookies and cake		37.8	0.0	0.0	1.8	4.0	7.3
Sugar		1.3	4.2	8.5	15.6	24.1	33.9
	1.2						

Noteworthy are the following points: First, the frequent consumption of milk and eggs, of green and other vegetables, tomatoes, and of citrus and other fruits. Secondly, contrary to widespread impression of the dietary habits in this region, cornbread was eaten less than other forms of bread. Thirdly, although 1 to 85% of the patients were recorded as eating none of the individual foodstuffs listed, when these foods were grouped into the basic 7 categories, only 3.7% were recorded as eating no green leafy, green or yellow vegetables; 6.7% no tomatoes or citrus fruits; 0.4% no potatoes, other vegetables or fruits; 0.7% no milk, cheese or ice cream; 0.1% no meat, poultry, fish, eggs or legumes; 0.1% no bread, flour or cereals; and 12.5% no butter or margarine. The diets, therefore, were gratifyingly varied.

Pharmaceutical supplements are not routinely prescribed in our obstetric clinic and the usual practice was not altered during this study. Seventy-three per cent of our subjects received no dietary supplements, 14% were given prescriptions for calcium, 10% for iron, 5% for a vitamin B complex mixture,[5] and 4% took various other types of supplements. Some patients received more than one type of preparation and so are included in more than one of the above percentage tabulations. The period of supplementation varied but seldom extended throughout the whole period of gestation. In calculating nutrient intakes, the contribution of pharmaceutical supplements has not been included.

Laboratory measurements

The laboratory methods employed have been described in a preceding report (Darby et al., '53). The determinations were carried out once during each trimester and at approximately the 6th week post partum. The data may conveniently be presented in the following groups (tables 5–8): (a) hematologic and serum protein values, (b) fat-soluble vitamins (vitamin A, carotene, tocopherols) in the serum or plasma, (c)

[5] The B complex syrup prescribed contained 25 mg of niacin, 1 mg of riboflavin and 3 mg of thiamine hydrochloride per 4 ml. Usually 4 ml three times per day were prescribed.

TABLE 5 [1]

Measures of central tendency and variability of laboratory determinations by weeks of gestation and weeks post partum: hematology

	WEEKS GESTATION OR POST PARTUM		NO. OF CASES	HEMOGLOBIN (GM/100 ML)			RED BLOOD COUNT (MILLIONS/MM³)			PACKED CELL VOLUME (%)		
	Range	Median		Mean ± S.E.	S.D.	Range of mean ± 2 S.D.	Mean ± S.E.	S.D.	Range of mean ± 2 S.D.	Mean ± S.E.	S.D.	Range of mean ± 2 S.D.
(Patients who entered the clinic during the first trimester only)												
First trimester	5–9	8.8	131	13.13 ± 0.12	1.37	10.4–15.9	4.38 ± 0.04	0.45	3.5–5.3	39.2 ± 0.3	3.4	32.4–46.0
	10–13	11.8	206	12.95 ± 0.08	1.15	10.6–15.3	4.29 ± 0.03	0.45	3.4–5.2	38.5 ± 0.2	3.1	32.3–44.6
Second trimester	14–19	18.9	103	12.01 ± 0.10	1.06	9.9–14.1	4.04 ± 0.04	0.44	3.2–4.9	35.8 ± 0.3	3.0	29.9–41.7
	20–26	21.5	184	11.94 ± 0.08	1.09	9.8–14.1	3.98 ± 0.03	0.45	3.1–4.9	35.5 ± 0.2	2.8	29.9–41.1
Third trimester	27–32	31.3	220	11.43 ± 0.09	1.30	8.8–14.0	3.88 ± 0.03	0.41	3.1–4.7	34.8 ± 0.2	3.4	28.0–41.6
	33 and over	34.1	72	11.55 ± 0.17	1.43	8.7–14.4	4.08 ± 0.06	0.49	3.1–5.1	35.1 ± 0.4	3.5	28.2–42.1
Post partum	under 6	5.4	42	12.66 ± 0.23	1.52	9.6–15.7	4.48 ± 0.06	0.39	3.7–5.2	39.6 ± 0.5	3.5	32.7–46.5
	6 and over	6.9	216	13.09 ± 0.09	1.26	10.6–15.6	4.55 ± 0.03	0.46	3.6–5.5	40.5 ± 0.2	2.9	34.7–46.3

	WEEKS GESTATION OR POST PARTUM		NO. OF CASES	MEAN CELL VOLUME (μ^3)			MEAN CELL HEMOGLOBIN ($\mu\mu G$)			MEAN CELL HEMOGLOBIN CONCENTRATION (%)		
	Range	Median		Mean ± S.E.	S.D.	Range of mean ± 2 S.D.	Mean ± S.E.	S.D.	Range of mean ± 2 S.D.	Mean ± S.E.	S.D.	Range of mean ± 2 S.D.
(All cases, regardless of time of entry)												
First trimester	5–9	8.8	131	89.8 ± 0.7	7.5	75–105	30.04 ± 0.25	2.92	24.2–35.9	33.42 ± 0.17	1.97	29.5–37.4
	10–13	11.8	206	90.5 ± 0.6	9.1	72–109	30.43 ± 0.22	3.12	24.2–36.7	33.75 ± 0.13	1.82	30.1–37.4
Second trimester	14–19	17.1	674	89.5 ± 0.3	7.7	74–105	30.11 ± 0.12	3.01	24.1–36.1	33.66 ± 0.07	1.80	30.1–37.3
	20–26	22.6	691	89.9 ± 0.3	8.0	74–106	30.09 ± 0.12	3.14	23.8–36.4	33.45 ± 0.07	1.93	29.6–37.3
Third trimester	27–32	30.9	1,395	90.1 ± 0.2	8.2	74–106	29.76 ± 0.09	3.27	23.2–36.3	33.05 ± 0.05	2.00	29.0–37.1
	33 and over	34.6	562	88.3 ± 0.4	8.9	71–106	29.02 ± 0.15	3.58	21.9–36.2	32.74 ± 0.09	2.07	28.6–36.9
Post partum	under 6	5.4	241	88.0 ± 0.5	8.3	71–105	28.28 ± 0.22	3.44	21.4–35.2	32.08 ± 0.11	1.69	28.7–35.5
	6 and over	7.0	1,279	89.2 ± 0.2	8.6	72–106	28.81 ± 0.09	3.33	22.2–35.5	32.27 ± 0.05	1.82	28.6–35.9

[1] Throughout these tables the number of cases varied slightly for the different determinations. The number given is the minimum number for the group. S.E. = standard error of the mean; S.D. = standard deviation.

TABLE 6

Measures of central tendency and variability of laboratory determinations by weeks of gestation and weeks post partum: serum proteins

	WEEKS GESTATION OR POST PARTUM		NO. OF CASES	TOTAL SERUM PROTEIN (GM/100 ML)		
	Range	Median		Mean ± S.E.	S.D.[1]	Range of mean ± 2 S.D.
	(Patients who entered the clinic during the first trimester only)					
First trimester	5–9	8.8	132	6.95 ± 0.04	0.44	6.1–7.8
	10–13	11.8	206	6.87 ± 0.03		6.0–7.8
Second trimester	14–19	18.9	103	6.36 ± 0.04	0.40	5.6–7.2
	20–26	21.5	185	6.30 ± 0.03		5.5–7.1
Third trimester	27–32	31.3	219	6.24 ± 0.03	0.41	5.4–7.1
	33 and over	34.1	71	6.35 ± 0.05		5.5–7.2
Post partum	under 6	5.4	40	7.00 ± 0.07	0.42	6.2–7.8
	6 and over	6.9	211	7.15 ± 0.03	0.48	6.2–8.1

[1] The standard deviation was computed from the average variance within subgroups.
[2] Serum albumin and globulin values are based on determinations done after January 1, 1948, only.

TABLE 7

Measures of central tendency and variability of laboratory determinations by weeks of gestation and weeks post partum: lipid-soluble vitamins

	WEEKS GESTATION OR POST PARTUM		NO. OF CASES	SERUM VITAMIN A (I.U./100 ML) (all cases)			WEEKS GESTATION OR POST PARTUM	
	Range	Median		Mean ± S.E.	S.D.[1]	Range of mean ± 2 S.D.	Range	Median
First trimester	5–9	8.8	130	111.1 ± 2.6	29.4	52–170	5–9	8.8
	10–13	11.8	202	112.0 ± 2.1		53–171	10–13	11.8
Second trimester	14–16	15.4	318	114.6 ± 1.6		56–173	14–19	18.9
	17–19	18.6	343	115.2 ± 1.6		56–174		
	20–22	21.4	396	113.3 ± 1.5		55–172	20–26	21.5
	23–26	24.6	290	105.9 ± 1.7		47–165		
Third trimester	27–29	28.5	450	101.9 ± 1.4		43–161	27–32	31.3
	30–32	31.6	932	101.1 ± 1.0		42–160		
	33–35	34.0	401	96.9 ± 1.5		38–156	33 and over	34.1
	36 and over	37.6	152	98.8 ± 2.4		40–158		
Post partum	under 6	5.4	237	144.9 ± 2.4	37.6	70–220	under 6	5.4
	6 and over	7.0	1256	139.2 ± 1.0	37.0	65–213	6 and over	6.9

[1] The standard deviation was computed from the average variance within subgroups.

TABLE 6

Measures of central tendency and variability of laboratory determinations by weeks of gestation and weeks post partum: serum proteins

WEEKS GESTATION OR POST PARTUM		NO. OF CASES	SERUM ALBUMIN (GM/100 ML)[2]			SERUM GLOBULIN (GM/100 ML)[2]		
Range	Median		Mean ± S.E.	S.D.	Range of mean ± 2 S.D.	Mean ± S.E.	S.D.	Range of mean ± 2 S.D.
(Patients who entered the clinic during the first trimester only)								
5–13	10.6	83	4.42 ± 0.04	0.34	3.7–5.1	2.54 ± 0.05	0.46	1.6–3.5
14–19	18.9	35	3.99 ± 0.05	0.28	3.4–4.5	2.37 ± 0.07	0.42	1.5–3.2
20–26	21.1	52	4.06 ± 0.04	0.27	3.5–4.6	2.26 ± 0.05	0.36	1.5–3.0
27–32	31.1	88	3.87 ± 0.03	0.28	3.3–4.4	2.36 ± 0.04	0.41	1.5–3.2
33 and over	34.3	20	3.88 ± 0.05	0.23	3.4–4.3	2.62 ± 0.10	0.43	1.8–3.5
under 6	5.4	14	4.63 ± 0.07	0.28	4.1–5.2	2.41 ± 0.12	0.43	1.5–3.3
6 and over	6.9	98	4.57 ± 0.03	0.30	4.0–5.2	2.61 ± 0.05	0.51	1.6–3.6

TABLE 7

Measures of central tendency and variability of laboratory determinations by weeks of gestation and weeks post partum: lipid-soluble vitamins

NO. OF CASES	SERUM CAROTENE (μG/100 ML) Entered first trimester only			WEEKS GESTATION OR POST PARTUM		NO. OF CASES	PLASMA TOCOPHEROL (MG/100 ML) (all cases)		
	Mean ± S.E.	10th Percentile	90th Percentile	Range	Median		Mean ± S.E.	S.D.	Range of mean ± 2 S.D.
131	114.4 ± 3.8	60	164	under 14	11.5	74	0.89 ± 0.024	0.20	0.48–1.30
203	118.3 ± 3.1	60	180						
103	149.7 ± 5.1	88	220	14–18	16.5	240	1.04 ± 0.015	0.23	0.57–1.51
184	157.6 ± 3.7	92	224	19–22	21.0	258	1.09 ± 0.014	0.23	0.63–1.55
				23–26	25.0	273	1.16 ± 0.015	0.24	0.68–1.64
221	172.6 ± 4.7	88	264	27–30	29.0	273	1.21 ± 0.016	0.26	0.68–1.74
72	172.8 ± 8.3	80	260	31–34	33.0	276	1.26 ± 0.015	0.25	0.76–1.76
				35–38	37.0	149	1.32 ± 0.024	0.29	0.73–1.91
				39–46	41.0	32	1.40 ± 0.050	0.28	0.83–1.97
41	119.5 ± 7.4	56	180						
213	122.0 ± 3.5	60	188						

TABLE 8

Measures of central tendency and variability of laboratory determinations by weeks of gestation and weeks post partum: water-soluble factors
(All cases, regardless of trimester of entry)

	WEEKS GESTATION OR POST PARTUM		NO. OF CASES	SERUM VITAMIN C (MG/100 ML)			WEEKS GESTATION OR POST PARTUM		NO. OF CASES	THIAMINE EXCRETION (MG/2 HR.)[1]			N′-METHYLNICOTINAMIDE (MG/2 HR.)[1]		
	Range	Median		Median	10th Percentile	90th Percentile	Range	Median		Median	10th Percentile	90th Percentile	Median	10th Percentile	90th Percentile
First trimester	5–9	8.8	125	0.50	0.08	1.12	5–9	8.8	61	0.12	0.03	0.31	5.25	2.62	11.18
	10–13	11.8	195	0.45	0.10	1.07	10–13	11.7	118	0.13	0.06	0.27	6.45	2.98	11.28
Second trimester	14–16	15.4	323	0.53	0.10	1.11	14–16	15.4	182	0.12	0.05	0.30	6.98	2.85	12.85
	17–19	18.6	337	0.49	0.10	1.12	17–19	18.6	236	0.11	0.03	0.26	6.92	2.82	13.60
	20–22	21.4	389	0.46	0.09	1.00	20–22	21.4	250	0.11	0.04	0.22	7.37	3.05	14.98
	23–26	24.6	287	0.39	0.07	0.96	23–26	24.7	169	0.10	0.03	0.20	7.98	3.28	16.95
Third trimester	27–29	28.5	457	0.34	0.06	0.94	27–29	28.7	301	0.10	0.02	0.22	9.02	3.60	18.62
	30–32	31.6	917	0.36	0.06	0.96	30–32	31.5	604	0.08	0.02	0.17	9.00	3.30	17.28
	33–35	34.0	399	0.34	0.06	0.94	33–35	34.0	181	0.07	0.01	0.16	8.72	3.45	16.22
	36 and over	37.6	124	0.35	0.06	0.84	36 and over	37.4	70	0.08	0.02	0.22	8.82	3.40	21.25
Post partum	under 6	5.4	224	0.20	0.00	0.76	under 6	5.4	167	0.10	0.02	0.23	5.78	2.68	10.78
	6 and over	7.0	1,140	0.20	0.00	0.76	6 and over	6.9	719	0.09	0.02	0.22	4.95	2.00	9.28

[1] Thiamine and N′-methylnicotinamide exeretions are for patients who entered the clinic after January 1, 1947.

serum ascorbic acid levels, and (d) urinary excretion of B vitamins following load tests.

The trends occurring during pregnancy and post partum are most easily presented in graphic form (figs. 1, 2, 3).

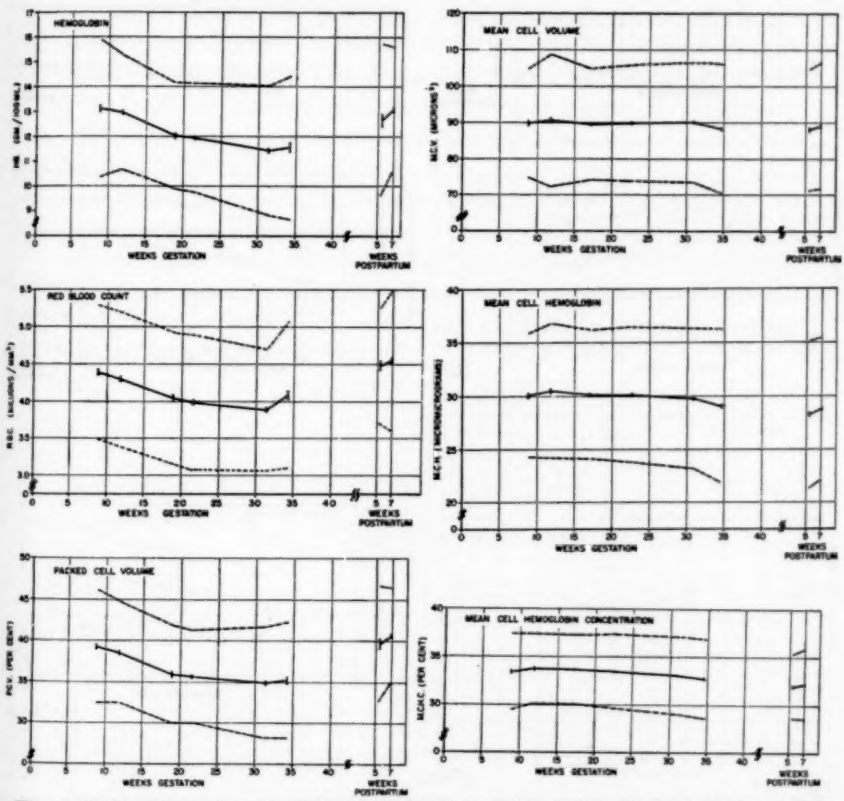

Fig. 1 Laboratory values according to weeks of gestation and weeks post partum: hematology.

Solid line shows trend of mean values; short vertical lines through each mean value extend a distance of one standard error of the mean on either side; dotted lines represent the mean ± two standard deviations.

Vertical scale unit is proportional to 4% of the mean value at 5 to 9 weeks of gestation.

Values are plotted at the median week's gestation for each interval (see table 5).

Hb, RBC and PCV trends are based on cases who entered the clinic during the first trimester of pregnancy; MCV, MCH and MCHC trends are based on all cases, regardless of the trimester of entry.

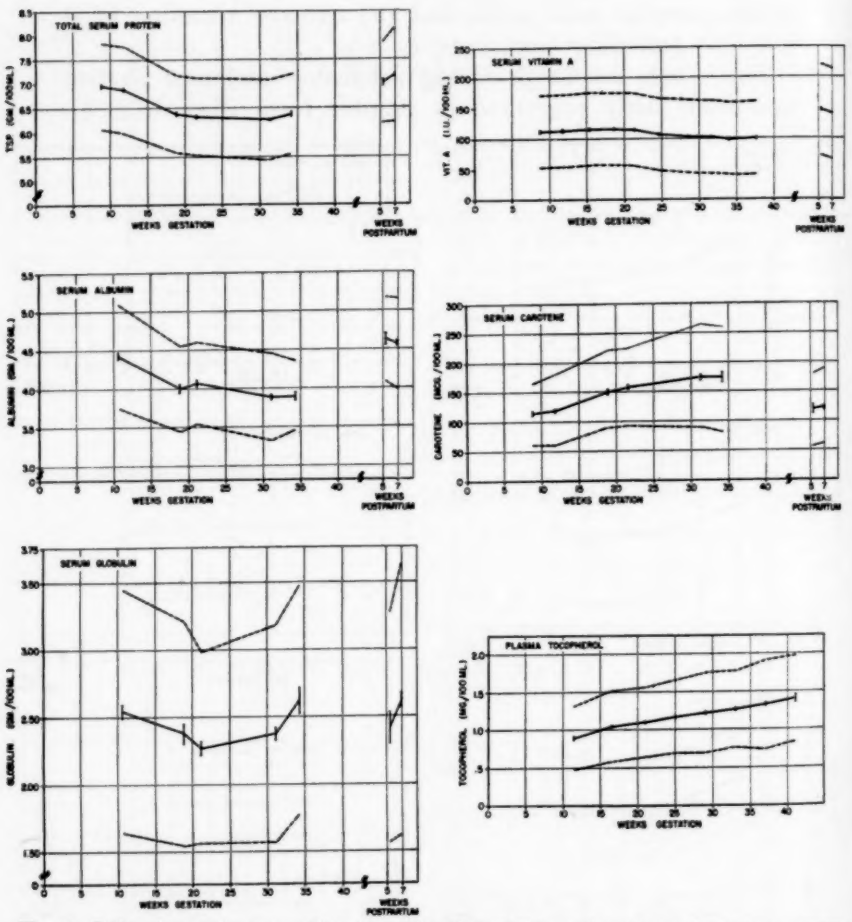

Fig. 2 Laboratory values according to weeks of gestation and weeks post partum: serum proteins and fat-soluble vitamins.

Solid line shows trend of mean values; short vertical lines through each mean value extend a distance of one standard error of the mean on either side. For all determinations except carotene, dotted lines represent the mean $\pm$ two standard deviations; for carotene the dotted lines represent the 10th and 90th percentiles.

TSP, albumin, globulin and carotene trends are based on cases who entered the clinic during the first trimester of gestation; in addition, for albumin and globulin only determinations done after January 1, 1948, were included. Vitamin A and tocopherol are based on all cases.

Where the distribution is normal, the graphs depict the mean with its standard error and the limits of two standard deviations on either side of the mean; where the distribution is skewed, the trends depicted are those for the 10th, 50th (median) and 90th percentiles. For carotene the data are moderately skewed, and the mean and the 10th and 90th percentiles are shown. The composition of the groups described for these estimations varies somewhat, due to the necessity of eliminating the effects upon the distributions of long-term trends or other factors.

Since the impression of the magnitude of the changes in laboratory values is dependent upon the size of the unit of the vertical scale, the series of graphs have been prepared on an as nearly comparable basis as was feasible. It should be noted that this scale is usually broken between the lowest

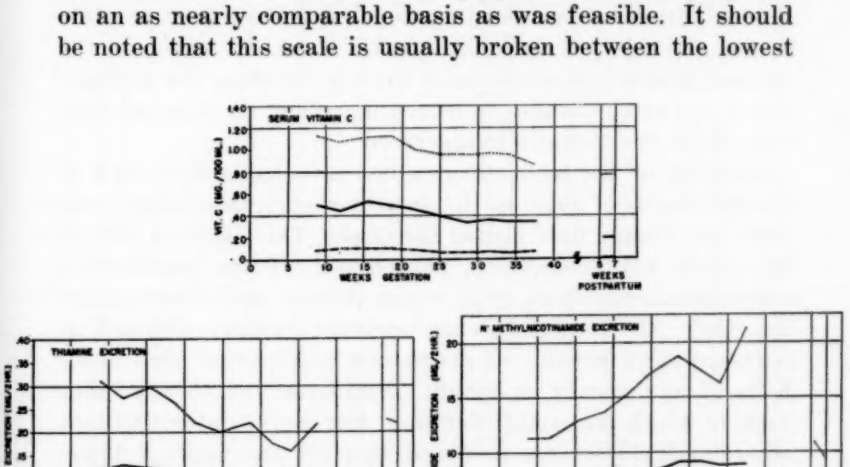

Fig. 3 Laboratory values according to weeks of gestation and weeks post partum: water-soluble factors.

Solid line shows median value, dotted lines the 10th and 90th percentiles.

Vertical scale unit is proportional to 25% of median value at 5 to 9 weeks of gestation.

Trend for vitamin C is based on all cases; for thiamine and N'-methylnicotinamide on cases who entered the study after January 1, 1947.

values and zero. The variabilities about the mean or median values and the changes in the values are shown in proportion to the earliest (5th to 9th week) determinations in the first trimester by selection of the vertical scale units as follows: (1) For hematologic values and serum proteins, a fixed vertical distance represents 4% of the mean observations at the 5- to 9-week period of gestation. (2) For vitamin A, serum carotene and tocopherol, this vertical distance is adjusted to represent 20% of the initial mean values. (3) For serum ascorbic acid and for the data on urinary excretion of the B vitamins, the same distance represents 25% of the median of the initial estimations.

The variability of determinations in groups 2 and 3 was so much greater than that in group 1 that it was not possible to use a single proportionate scale for all. In group 1, the vertical scales are necessarily broken between the average levels and zero; changes in levels, therefore, on a percentage-wise basis, are visually exaggerated.

Analysis of the laboratory values revealed a difference in average levels of some of the measurements, depending upon when the woman first visited the clinic. This effect is seen in the values for hemoglobin, packed cell volume, erythrocyte count, serum carotene, total serum protein and serum globulin (table 9). This difference occurred in data obtained at corresponding periods of pregnancy and was not due to effects of age, parity or weight distribution, or to any other factors which we could identify. For those determinations where such differences occurred, figure 1 and figure 2 depict the trends for the cases who entered the clinic during the first trimester. Differences in the average level according to trimester of entry are shown in figure 4. For other values, the trend is based on all cases.

All of the laboratory determinations were examined for evidence of long-term trends in the values during the period of the study. Such trends were apparent in the data on the urinary excretion of thiamine, riboflavin and N′-methylnicotinamide and on the concentration of albumin in the serum.

TABLE 9

Mean ± standard error for certain laboratory determinations by weeks of gestation and weeks post partum for patients who entered the clinic in the second and third trimesters

	WEEKS GESTATION OR POST PARTUM	MEDIAN WEEKS	NO. OF CASES	Hb	RBC	PCV	SERUM CAROTENE	TOTAL SERUM PROTEIN	MEDIAN WEEKS	NO. OF CASES	ALBUMIN [1]	GLOBULIN [1]
				gm/100 ml	*millions/mm³*	*%*	*μg/100 ml*	*gm/100 ml*			*gm/100 ml*	*gm/100 ml*
Patients who entered the clinic in the second trimester												
Second trimester	14–19	16.6	566	12.51 ± 0.05	4.17 ± 0.02	37.1 ± 0.1	139.3 ± 2.2	6.67 ± 0.02	17.1	176	4.20 ± 0.02	2.50 ± 0.03
	20–26	23.0	501	12.05 ± 0.05	4.04 ± 0.02	36.2 ± 0.1	148.6 ± 2.5	6.51 ± 0.02	22.8	143	4.00 ± 0.02	2.53 ± 0.04
Third trimester	27–32	31.3	718	11.53 ± 0.05	3.90 ± 0.01	34.8 ± 0.1	164.8 ± 2.4	6.26 ± 0.01	31.2	290	3.89 ± 0.02	2.41 ± 0.03
	33 and over	34.0	244	11.49 ± 0.09	3.96 ± 0.03	34.9 ± 0.2	160.4 ± 3.6	6.24 ± 0.02	34.2	70	3.81 ± 0.04	2.42 ± 0.05
Post partum	under 6	5.4	145	12.88 ± 0.11	4.60 ± 0.04	40.0 ± 0.3	127.6 ± 4.8	7.10 ± 0.04	5.4	86	4.53 ± 0.03	2.61 ± 0.05
	6 and over	6.9	698	12.95 ± 0.05	4.55 ± 0.02	40.3 ± 0.1	119.7 ± 2.0	7.14 ± 0.02	6.9	304	4.61 ± 0.02	2.59 ± 0.03
Patients who entered the clinic in the third trimester												
Third trimester	27–32	29.3	448	11.95 ± 0.06	4.04 ± 0.02	36.2 ± 0.2	148.4 ± 2.8	6.49 ± 0.02	29.1	133	3.96 ± 0.03	2.50 ± 0.04
	33 and over	35.7	236	11.90 ± 0.10	4.14 ± 0.03	36.3 ± 0.2	154.8 ± 4.1	6.51 ± 0.03	36.0	59	3.88 ± 0.04	2.58 ± 0.06
Post partum	under 6	5.4	52	12.92 ± 0.20	4.58 ± 0.06	40.2 ± 0.5	130.2 ± 5.9	7.13 ± 0.05	5.4	27	4.56 ± 0.05	2.59 ± 0.07
	6 and over	7.2	352	13.08 ± 0.07	4.55 ± 0.02	40.4 ± 0.2	113.3 ± 2.7	7.19 ± 0.03	7.0	133	4.60 ± 0.03	2.66 ± 0.04

[1] Serum albumin and globulin values are based on determinations done after January 1, 1948, only.

In the case of riboflavin there was a gradual upward trend in the median value from the early part of the study until early in 1948, at which time there was a leveling off and then a decrease (fig. 5). A review of the data, and study of the

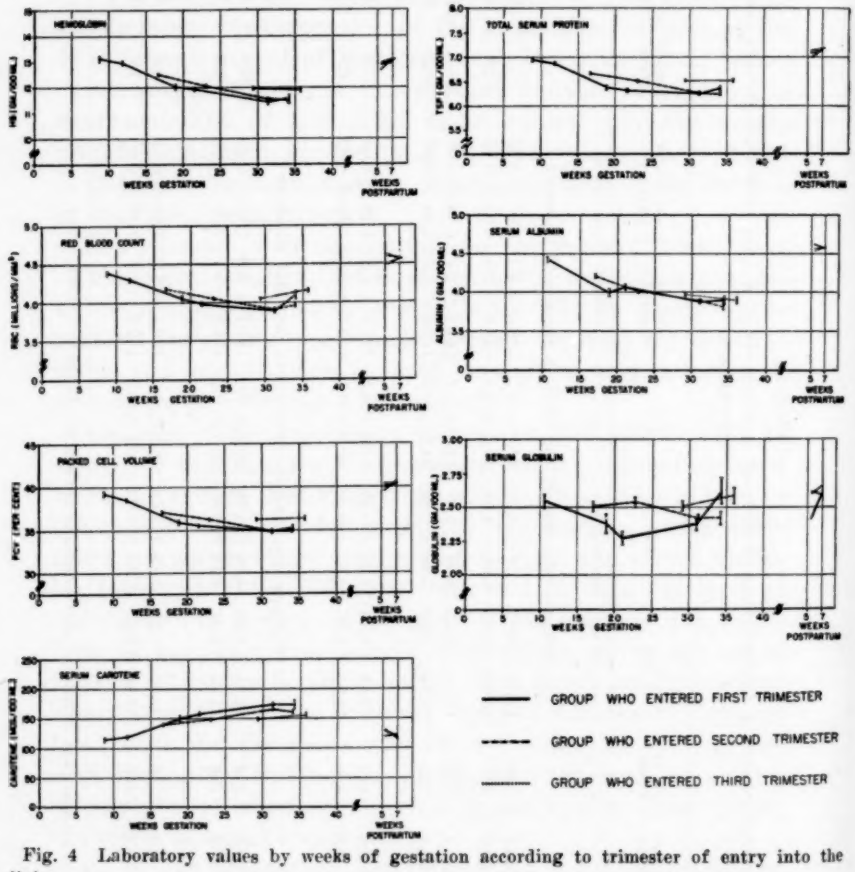

Fig. 4 Laboratory values by weeks of gestation according to trimester of entry into the clinic.

Lines show trends of mean values; short vertical lines through each mean extend a distance of one standard error of the mean on either side (omitted post partum).

Vertical scale unit is proportional to 4% of the mean value at 5 to 9 weeks of gestation except for carotene, where the scale unit is proportional to 20% of the mean value at 5 to 9 weeks.

Trends for albumin and globulin are based on determinations done after January 1, 1948.

original laboratory notes and of the standards and procedures used, did not reveal a cause for this trend. It is believed that it is intrinsic within the persons studied, in spite of the fact that similar trends in dietary intake were not apparent. The superimposition of a time trend onto the trend by weeks of gestation makes it difficult to give meaningful interpretation to the latter; in fact, different trends during gestation were obtained during different periods of the study. Figure 5 and table 10 show riboflavin excretion according to calendar date for specimens obtained during the second and third trimesters and post partum. The group of patients who entered the

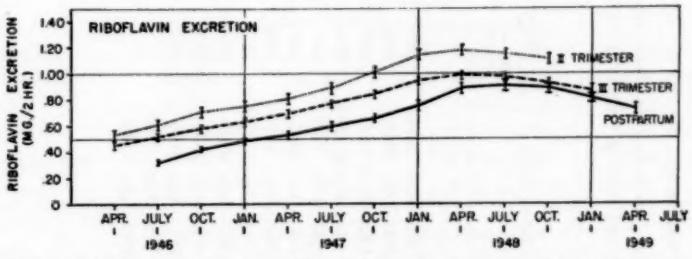

Fig. 5 Moving average of median riboflavin excretion values according to calendar date, second and third trimesters and post partum.

Cases who entered the clinic in the first trimester are not included.

Vertical scale unit is proportional to 25% of the median value at 5 to 9 weeks of gestation for the whole time period.

clinic during the first trimester have been omitted. The trends shown were obtained by taking the median value of the determinations by three-month periods and then computing a moving average of these medians by 4-quarter periods, in order to minimize the effect of season.

Thiamine and N′-methylnicotinamide excretion values were higher during the earliest portion of the study than later on, but had leveled off by the end of 1946 and remained stable from 1947 through 1949. Again, no explanation for this trend has been found. Data shown in figure 3 and table 8 are for the period 1947 to 1949. The excluded data are given in table 11.

TABLE 10

Riboflavin excretion values by calendar date for second and third trimesters[1] and post partum
(Median values by three-month periods, and moving average of medians, taken 4 at a time)

CALENDAR DATES	MEDIAN VALUES (MG/2 HR.)					
	Second trimester		Third trimester		Post partum	
	No. of cases	Median	No. of cases	Median	No. of cases	Median
Fall, 1945	52	0.60	65	0.40		
Winter, 1946	72	0.49	135	0.45	33	0.29
Spring, 1946	94	0.40	141	0.44	85	0.23
Summer, 1946	62	0.60	88	0.51	60	0.27
Fall, 1946	76	0.93	105	0.66	65	0.48
Winter, 1947	103	0.87	137	0.69	86	0.69
Spring, 1947	90	0.56	135	0.64	78	0.47
Summer, 1947	76	0.84	126	0.76	115	0.45
Fall, 1947	79	1.24	125	0.94	90	0.74
Winter, 1948	81	1.36	117	0.98	87	0.96
Spring, 1948	75	1.10	121	1.06	70	0.85
Summer, 1948	74	0.99	116	0.93	87	0.97
Fall, 1948	65	1.13	99	0.91	101	0.84
Winter, 1949	34	1.20	75	0.77	82	0.86
Spring, 1949			29	0.87	66	0.55
Summer, 1949					33	0.64

CALENDAR DATES	MOVING AVERAGE ± S.E.					
	Second trimester		Third trimester		Post partum	
	No. of cases	Average ± S.E.	No. of cases	Average ± S.E.	No. of cases	Average ± S.E.
Oct. 1945–Sept. 1946	280	0.52 ± 0.04	429	0.45 ± 0.03		
Jan. 1946–Dec. 1946	304	0.61 ± 0.04	469	0.51 ± 0.03	243	0.32 ± 0.02
Apr. 1946–Mar. 1947	335	0.70 ± 0.04	471	0.57 ± 0.03	296	0.42 ± 0.02
July, 1946–June, 1947	331	0.74 ± 0.04	465	0.62 ± 0.03	289	0.48 ± 0.02
Oct. 1946–Sept. 1947	345	0.80 ± 0.04	503	0.69 ± 0.03	344	0.52 ± 0.03
Jan. 1947–Dec. 1947	348	0.88 ± 0.04	523	0.76 ± 0.03	369	0.59 ± 0.03
Apr. 1947–Mar. 1948	326	1.00 ± 0.04	503	0.83 ± 0.03	370	0.65 ± 0.03
July, 1947–June, 1948	311	1.14 ± 0.04	489	0.94 ± 0.03	362	0.75 ± 0.04
Oct. 1947–Sept. 1948	309	1.17 ± 0.04	479	0.98 ± 0.03	334	0.88 ± 0.04
Jan. 1948–Dec. 1948	295	1.15 ± 0.04	453	0.97 ± 0.03	345	0.90 ± 0.04
Apr. 1948–Mar. 1949	248	1.11 ± 0.04	411	0.91 ± 0.03	340	0.88 ± 0.04
July, 1948–June, 1949			319	0.87 ± 0.04	336	0.80 ± 0.03
Oct. 1948–Sept. 1949					282	0.72 ± 0.04

[1] Cases who entered the clinic in the first trimester are not included.

TABLE 11

Summary of laboratory determinations excluded from tables 6 and 8

	WEEKS GESTATION OR POST PARTUM	NO. OF CASES	MEAN ± S.E.	
			Albumin	Globulin
			gm/100 ml	
Serum albumin and globulin determinations done in 1945–1947				
Cases who entered clinic in first trimester				
First trimester	5–13	225	4.60 ± 0.03	2.28 ± 0.04
Second trimester	14–19	65	4.25 ± 0.04	2.11 ± 0.06
	20–26	121	4.15 ± 0.03	2.14 ± 0.04
Third trimester	27–32	118	4.05 ± 0.04	2.18 ± 0.05
	33 and over	45	4.00 ± 0.06	2.27 ± 0.07
Post partum	under 6	21	4.66 ± 0.10	2.33 ± 0.10
	6 and over	91	4.73 ± 0.05	2.34 ± 0.05
Cases who entered clinic in second trimester				
Second trimester	14–19	356	4.41 ± 0.02	2.25 ± 0.02
	20–26	317	4.25 ± 0.02	2.23 ± 0.02
Third trimester	27–32	386	4.08 ± 0.02	2.16 ± 0.02
	33 and over	151	4.07 ± 0.03	2.18 ± 0.04
Post partum	under 6	58	4.62 ± 0.05	2.40 ± 0.07
	6 and over	345	4.73 ± 0.02	2.33 ± 0.03
Cases who entered clinic in third trimester				
Third trimester	27–32	292	4.27 ± 0.02	2.23 ± 0.02
	33 and over	158	4.14 ± 0.03	2.39 ± 0.04
Post partum	under 6	23	4.68 ± 0.07	2.46 ± 0.08
	6 and over	201	4.80 ± 0.02	2.36 ± 0.03

	WEEKS GESTATION OR POST PARTUM	NO. OF CASES	MEDIAN VALUE	
			Thiamine	N′-methylnicotinamide
			mg/2 hr.	
Thiamine and N′-methylnicotinamide excretions for cases entering study in 1945–1946				
First trimester	5–9	63	0.14	7.04
	10–13	77	0.14	7.55
Second trimester	14–16	140	0.14	8.06
	17–19	101	0.12	9.19
	20–22	139	0.11	9.54
	23–26	118	0.12	11.20
Third trimester	27–29	155	0.11	10.60
	30–32	312	0.09	11.04
	33–35	216	0.09	11.25
	36 and over	53	0.09	10.62
Post partum	under 6	57	0.09	5.86
	6 and over	421	0.11	5.44

There were small but statistically significant trends in the mean serum albumin values. These trends were not associated with similar changes in the concentration of total serum protein. Slightly higher mean values and greater variability in the serum albumin were recorded prior to January, 1948. This has been attributed to variation among several laboratory technicians who were doing the determinations prior to that time. Subsequent to January, 1948, all determinations were made by one worker and the stability of mean values after this date was maintained. Data shown are for the period after January 1, 1948 (table 6). These data vary but little from those obtained prior to January 1, 1948 (table 11). The problems of maintaining uniform laboratory standards, of early detection of any long-term trend in the data — whether due to laboratory variation or to real changes in the patients — and of evaluating the data where such trends existed, merit special consideration. In long-term nutrition studies this is an important problem, and it will be discussed in a projected critique of methodology arising from this investigation.

Hematology and serum proteins. The expected decrease in hemoglobin concentration, erythrocyte count and packed cell volume occurred during pregnancy. The means and two standard deviation limits of the observations are listed in table 5. These decreases in average values during pregnancy were not associated with changes in the blood indices to an extent that would suggest that the development was due mainly to either an iron deficiency or a lack of one of the hemopoietic vitamins. Instead, we believe that this normochromic, normocytic change reflects primarily the increased plasma volume (hemodilution) of pregnancy. The extent of this physiologic anemia of pregnancy has not been appreciated by all workers who have studied nutrition in pregnancy.

The post-partum hemotologic values are of interest in that the average erythrocyte count and packed cell volume (PCV) returned to levels somewhat higher than the values observed at the 5th to 9th week of gestation, whereas the hemoglobin

barely regained its maximum concentration. This results in a further small decrease in the mean values for mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) at the 6-week post-partum examination. Differences in the average mean cell volume (MCV) between the third trimester and post partum are within the range of chance variation. All of these findings indicate some barely perceptible limitation in hemopoiesis due to iron deficiency, possibly beginning in the third trimester and revealing itself definitely in the post-partum period. This is largely a result of blood loss associated with the actual delivery or during the period of the puerperium. The magnitude of these average changes are such that they are not likely to be recognizable in the study of an individual patient, because they are small relative to the error of an individual determination. They may be detected only in the average values of large groups such as this.

Inspection of the graphs reveals a decrease in the concentration of serum albumin, which is consistent with the view that this change reflects primarily the common physiological mechanism of an increased plasma volume or hemodilution.

It is of interest that the serum globulin [6] fraction rose during the latter part of gestation, following an initial decrease. Other workers (Rinehart, '45; Coryell et al., '50; Wiehl, '50) have found an increase in the serum globulin in pregnancy but not the initial drop that was observed in our series. This drop is statistically significant. However, for the group who entered the clinic in the second trimester there was a decrease rather than a rise in the serum globulin levels between the second and third trimesters.

The observation that during the second and third trimesters the hemoglobin and erythrocyte levels are higher in those women attending the clinic for the first time than in those who sought prenatal care earlier is not easy to explain. As noted

[6] For the purposes of this study, the levels of serum globulin were determined by subtracting the serum albumin level from that of the total serum protein.

above, this effect is not attributable to differences in age, parity, initial weight of the mother, or seasonal distribution. One explanation may be that a factor which influences a woman to seek prenatal care early is a vague, ill-defined sense of less-than-bouyant health during the earlier months of pregnancy and that this is associated in many instances with depressed hematologic values. This is but a surmise and unfortunately cannot be explored by our data. This hypothesis is attractive, however, in view of the slightly lower hematologic values and dietary intakes of the women who sought care early in their pregnancy. That it may not be the sole explanation, however, is suggested by the fact that certain of the values for the third trimester became identical for those women who entered during the first and second trimesters.

Serum ascorbic acid. Serum ascorbic acid concentration shows on the average a decrease during gestation, followed by a further decline by the 6th week post partum. Especially low ascorbic acid values occurred in lactating women. Mothers who sublet their duty to a cow showed a smaller post-partum decline in serum ascorbic acid level. Serum vitamin C levels of the distribution occurring here during pregnancy would, if encountered in a non-pregnant group of adults, occasion no grave concern as to their nutritional level. One would expect the population to be protected from clinically manifest deficiency disease due to lack of ascorbic acid.

Fat-soluble vitamins. Low values of serum vitamin A are much more frequently encountered in pregnant women than in the non-pregnant members of a population. In the present series the mean serum vitamin A values decreased during pregnancy and then rose. These post-partum values for serum vitamin A and carotene were higher than the levels observed for non-pregnant, non-lactating women in an adjacent county in 1944 (Darby et al., '53) and the vitamin A concentrations were greater than those observed in a North Carolina population in 1943–1944 (Darby and Milam, '45). On the other hand, the carotene levels in the North Carolina

population were, when expressed in micrograms, similar for the white women to those which we obtained in the present study. The decrease in concentration of vitamin A in the serum during pregnancy is a recognized metabolic phenomenon (Darby, Cannon and Kaser, '48), and again points to the necessity for understanding clearly the expected physiological changes in the level of a nutrient before passing judgment upon the nutriture of a patient, based upon the result of a biochemical measurement.

Serum values of both carotene and tocopherols rise during pregnancy. The degree of change in these two substances is approximately the same. This similarity of behavior for these two nutrients has been commented on previously (Darby, Cannon and Kaser, '48), and it has been noted that the rise is suggestively parallel with that which is known to occur in the concentration of cholesterol during gestation. Such observations are consistent with the hypothesis that these lipid-soluble substances are transported by similar mechanisms and that the lipid transport power of the serum is increased during pregnancy. At any rate, these increases reflect metabolic phenomena, not a change in dietary intake, for the latter was not increased during gestation.

B-Vitamin excretions. A downward trend in relation to week of gestation occurred in the median thiamine excretion following an oral test dose, but this trend was not great. By contrast, there was an early increase in N′-methylnicotinamide, the median value late in pregnancy rising to approximately twice that observed at the time of the earliest study or at the post-partum examination (fig. 3). The progressive rise in excretion of this metabolite occurred even in the 90th percentile, but in the 10th percentile the level of increase was relatively small, amounting to only 30%. Although the metabolic significance of these variations cannot be stated, it is apparent that use of this metabolite as a measure of nutriture can be explored only if one recognizes the existence of such phenomena (Darby, Cannon and Kaser, '48).

Riboflavin excretion was one of the measurements in which there occurred a gradual, progressive time trend (fig. 5). The magnitude of this time trend was even greater than the observed differences in excretion from one trimester to another. This increased urinary excretion from 1946 to 1948 was not a result of increases in visible riboflavin in the diet, although it may signify an accumulation of this nutrient in the stores of the patients. Our data on supplementation indicate that it did not reflect an increased intake as a result of vitamins consumed as pharmaceutical supplements. A likely explanation is that some unrecognized source of increased riboflavin was in the diet and was exerting a cumulative effect during this interval, or that the gradual change in the economic composition of our sample was reflected in an enhanced store of riboflavin. It may possibly be that such a dietary source was a small additional supply of the vitamin which had accrued to the intake through the consumption of enriched flour, bread and cereal products, and that the maximum effects were not apparent until near the end of this study.

Physical findings

Physical examinations of the prenatal patients were made during all three trimesters of pregnancy and on the postpartum visit. These examinations were in addition to the routine prenatal checks and were performed by members of the attending staff, residents and senior assistant residents, after a period of instruction and supervision. We had hoped to train all personnel to a uniform recognition of physical signs [7] of possible nutritional interest. The results are ample proof that one cannot regiment judgment. All examiners were in agreement on two features: (1) the recording of height and weight, these being objective measurements, was consistent; (2) not one examiner found a single case of clinically recognizable deficiency disease such as pellagra, beriberi or scurvy. However, in all of the so-called "subclinical signs"

[7] See figure 1 of Darby et al. ('53).

of deficiency there were gross variations from one examiner to another (Bean, '48). In analysis, therefore, these examiner differences must be considered in addition to the effects of age, parity, season and pregnancy trends. The multiplicity of these factors complicates the interpretation of the findings. The correlation of the findings with the nutrient and laboratory results and obstetrical performance have been investigated and will be discussed in succeeding papers.

In the 2,129 patients who had their prenatal care at the Vanderbilt University Hospital, the mean height was 64.0 inches. The weight distributions were skewed, with median levels as follows: first trimester, 122 lb.; second trimester, 127 lb.; third trimester, 139 lb,; and post partum, 126 lb.

There were 4 cases of symmetrical dermatitis on exposed surfaces, but they involved only the hands and lower forearm, and were not associated with oral or gastrointestinal symptoms. All responded to local therapeutic measures such as the removal of sensitizing substances (soaps, detergents, and so forth) and obviously were not of nutritional origin. The commonest nutritional disease was one of excess rather than deficiency; i.e., obesity. Obesity was recorded for 15.7% of all the women, varying from 11.5% during the first trimester to 19.3% during the third trimester of pregnancy.

Within the group of 2,129 patients attending our clinic for prenatal care there were 529 (24.8%) for whom was recorded no physical abnormality of any sort throughout their prenatal or puerperal period. The percentage incidence of physical signs by season, trimester, age and parity are tabulated in tables 12 and 13. The tabulations in table 12 are of all of the diagnoses recorded on any occasion during the period of observation. There were 1,021 women who were examined three times — second and third trimester and post partum. An appreciable percentage of these had one or more recorded abnormalities, but in only a small number were these abnormalities recorded on all three examinations. To illustrate, 21.5% had disorders of the eyes, 18.1% abnormalities of the skin and 24.2% varicose veins, on one or more

TABLE 12

Gross incidence of physical findings among women, by trimester and season[1]: all examiners

	I TRIMESTER (340 PATIENTS) % INCIDENCE BY					II TRIMESTER (1,372 PATIENTS) % INCIDENCE BY					III TRIMESTER (1,969 PATIENTS) % INCIDENCE BY					POST PARTUM (1,547 PATIENTS) % INCIDENCE BY				
	W	Sp	Su	F	Total	W	Sp	Su	F	Total	W	Sp	Su	F	Total	W	Sp	Su	F	Total
General nutriture																				
Satisfactory	76.0	76.7	75.0	79.0	76.8	76.8	75.9	81.7	73.8	77.0	69.3	74.7	76.8	76.7	74.1	70.5	76.2	79.9	76.8	75.9
Under	14.0	9.6	11.8	11.0	11.7	11.7	7.5	8.5	13.2	10.3	9.5	5.1	5.3	6.0	6.6	13.0	7.7	7.5	8.5	9.1
Obese	10.0	13.7	13.2	10.0	11.5	11.5	16.6	9.8	13.0	12.7	21.2	20.2	17.9	17.3	19.3	16.5	16.1	12.6	14.8	15.0
Skin																				
Normal	88.0	90.5	95.5	90.1	90.7	90.6	90.1	94.8	91.0	91.5	90.0	90.9	94.2	92.9	91.8	92.7	93.3	95.9	92.3	93.6
Dry	11.0	4.1	3.0	8.8	7.2	8.2	7.3	4.2	7.7	6.9	7.7	7.4	4.6	5.1	6.3	4.3	4.4	3.2	7.1	4.7
Others	1.0	5.4	1.5	1.1	2.1	1.2	2.6	1.0	1.3	1.6	2.3	1.7	1.2	2.0	1.9	3.0	2.3	0.9	0.6	1.7
Eyes																				
Normal	91.0	94.5	95.6	95.6	94.0	91.3	92.4	94.5	94.5	93.1	90.5	89.0	96.4	93.8	92.1	82.7	84.1	94.9	96.2	89.5
Pingueculae	6.0	1.4	2.9	3.3	3.6	5.1	3.2	3.3	3.4	3.8	6.4	5.3	2.2	2.1	4.2	9.2	5.4	2.7	1.4	4.6
Conjunctival injection	2.0	1.4	0.0	0.0	0.9	2.0	4.4	1.3	0.6	2.1	2.4	5.1	0.5	3.0	2.9	11.1	11.5	1.7	1.9	6.5
Others	2.0	2.7	1.5	1.1	1.8	2.8	2.9	1.6	1.8	2.3	1.1	2.7	1.7	1.7	1.8	4.1	6.1	1.5	0.8	3.1
Nose and mouth																				
Disorders	1.0	5.4	1.5	4.4	3.0	7.7	3.5	1.3	2.2	3.9	6.8	6.7	2.4	3.7	5.1	11.9	6.7	1.0	2.7	5.5
Gums																				
Normal	73.0	78.4	83.8	78.0	77.8	67.9	79.9	78.8	79.1	76.0	62.2	76.2	76.0	73.0	71.7	59.3	74.9	78.4	77.3	72.7
Red	23.0	16.2	10.3	14.3	16.5	26.8	13.1	14.3	13.8	17.5	33.0	15.4	16.6	17.2	21.0	36.3	20.2	15.3	17.0	22.0
Swollen	17.0	5.4	7.4	8.8	10.2	16.8	6.7	7.8	8.0	10.2	18.6	9.9	8.4	8.8	11.8	23.6	12.0	10.2	8.8	13.5
Pyorrhea	10.0	6.8	11.8	13.2	10.5	13.0	11.3	13.7	9.2	11.8	14.6	11.2	13.9	11.9	12.9	15.4	10.2	11.7	9.0	11.6
Tongue																				
Disorders	8.0	4.1	1.5	5.5	5.1	8.4	3.5	6.2	2.8	5.3	9.7	5.5	7.0	4.1	6.7	5.4	2.3	6.6	1.4	4.0
Lower extremities																				
Normal	84.8	94.6	92.6	90.1	90.1	81.4	85.5	86.6	86.5	84.8	71.0	74.0	79.4	82.6	76.4	87.8	91.6	91.5	89.0	90.0
Edema	4.0	2.7	1.5	1.1	2.4	9.7	2.9	3.6	2.2	4.8	17.0	11.2	9.1	4.7	10.8	4.9	2.6	1.5	0.0	2.2
Varicosities	12.1	4.1	5.9	8.8	8.1	12.2	12.8	11.1	12.3	12.1	17.7	19.0	14.8	15.2	16.9	7.9	7.7	7.8	11.0	8.5
Neurological																				
Disorders	2.7	7.0	0.0	0.0	3.3	2.0	2.8	1.2	2.3	1.5	3.0	5.3	2.1	1.9	2.2	1.7	3.8	0.8	0.7	1.6
Remarks																				
Night blindness	0.6	1.0	0.0	0.0	1.1	0.8	2.0	0.3	0.0	0.6	0.9	0.9	1.7	0.7	0.0	1.6	3.3	1.8	0.2	1.1

[1] W = winter, Sp = spring, Su = summer, F = fall.

TABLE 13

Percentage incidence of physical findings by age and parity in the third trimester

Age	Para 0	Para 1–2	Para 3+	Total
	NUMBER OF PHYSICAL EXAMINATIONS ON WHICH PER CENT FINDINGS ARE BASED			
< 20	351	118	0	469
20–29	242	557	246	1045
> 30	25	74	298	397
Total	618	749	544	1911

Per cent obesity findings

AGE	PARA 0	PARA 1–2	PARA 3+	TOTAL
< 20	11.1	16.9	...	12.6
20–29	12.4	18.0	23.2	17.9
> 30	36.0	20.3	32.9	30.7
Total	12.6	18.0	28.5	19.3

Per cent edema findings

AGE	PARA 0	PARA 1–2	PARA 3+	TOTAL
< 20	7.1	7.6	..	7.2
20–29	9.9	9.3	9.3	9.5
> 30	16.0	17.3	18.9	18.4
Total	8.6	9.9	14.5	10.8

Per cent skin findings

< 20	4.6	5.9	..	4.9
20–29	7.1	5.9	6.5	6.3
> 30	8.0	16.0	16.8	16.1
Total	5.7	6.9	12.1	8.0

Per cent neurological findings

< 20	2.6	2.5	..	2.6
20–29	2.5	3.1	4.5	3.3
> 30	0.0	2.7	2.4	2.3
Total	2.4	2.9	3.3	2.9

Per cent ocular findings

< 20	4.0	7.6	..	4.9
20–29	4.6	7.5	6.9	6.7
> 30	8.0	6.7	15.8	13.6
Total	4.4	7.5	11.8	7.7

Per cent varicose veins findings

< 20	3.4	6.8	..	4.3
20–29	7.4	13.6	26.8	15.3
> 30	0.0	29.3	39.1	34.8
Total	4.9	14.1	33.5	16.6

Per cent oral findings

< 20	2.6	2.5	..	2.6
20–29	3.3	3.4	4.1	3.5
> 30	4.0	6.7	9.8	8.8
Total	2.9	3.6	7.2	4.4

Per cent tongue findings

< 20	4.0	4.2	..	4.1
20–29	4.6	6.7	7.8	6.4
> 30	0.0	8.0	11.2	9.9
Total	4.1	6.4	9.7	6.6

Per cent teeth findings

< 20	0.3	0.0	..	0.2
20–29	1.2	2.9	3.7	2.7
> 30	4.0	20.3	13.2	13.9
Total	0.8	4.2	8.9	4.4

Per cent gingival findings

< 20	16.2	22.9	...	17.9
20–29	17.4	25.9	36.7	26.5
> 30	36.0	32.0	48.1	44.3
Total	17.5	26.0	43.0	28.1

W = winter, Sp = spring, Su = summer, F = fall.

examinations. But only one patient had ocular disorders recorded on all three examinations, only three patients had skin abnormalities, and 3.4% had varicosities recorded on each occasion.

Fifty-one per cent of the physical examinations were carried out by 5 examiners, while 10 observers were responsible for 77%. In certain diagnoses occurring with low frequency (such as mouth and ocular disorders) most of the diagnoses were recorded by one or two examiners. These examiner differences were responsible for the apparent trimester trends occurring in mouth disorders and ocular lesions. Of the 68 post-partum diagnoses of angular scars, 54 were made by one physician. It seems obvious that this examiner's criteria were different from those of the other examiners. With conjunctival injection there occurred small incidence during the first trimester (0.9%), with a maximum (6.5%) at the post-partum examination. One observer found conjunctival injection in 67 of the 150 post-partum cases he examined, while the remaining examiners found only 38 cases in 1,400 patients. In both situations the examiners saw the bulk of their cases during the winter and spring months.

Examiner differences were also responsible for the apparent seasonal variations noted in obesity and undernutrition; ocular, neurological and mouth disorders; and edema. Where the physical findings were frequently recorded by the examiners, the problem still remained. Instead of just one examiner's being responsible for the majority of the positive findings, there now occurred high, low and middle-of-the-road groups of examiners. For instance, pyorrhea was diagnosed 627 times (12.0%) in the total of 5,228 examinations. One examiner reported an incidence of 30.0% in 648 examinations, another found but 4.2% in 757 examinations, while yet another reported an incidence of 16.8% positive findings in 702 examinations. The diagnosis of obesity actually seemed to correlate inversely with the weight of the individual examiner. The physicians who recorded a low incidence of obesity were those individuals who might be judged to be

obese themselves or to have obese wives; the examiners recording a high incidence of obesity were the lean individuals with lean wives. These examples illustrate the great variation which occurs in the evaluation and diagnosis of the so-called "subclinical" signs of nutritional deficiency.

There are no means of fixing real values for such attributes. The decision as to diagnosis is subjective and reflects the whole background of the individual physician-observer. The fact that different observers record varying incidences of diagnoses produces a large doubtful zone where evidence of manifest "deficiency disease" is not overwhelming.

Seasonal variations, after adjusting for examiner and trimester relationships, were found in abnormalities affecting the skin (dry skin, keratosis pilaris, ichthyosis, and so forth). A definite decrease in these conditions occurred during the summer months. One would expect many dermatologic conditions to react in this manner. Gingival lesions, on the other hand, had an increase of borderline significance during the winter months. Ocular lesions, although small in number, occurred with greater frequency during the spring. Nose and mouth disorders were lowest in the summer, while edema was lowest in the fall as compared to a winter-spring maximum.

True pregnancy or trimester trends were seen in the recording of undernutrition. This was least frequently recorded during the third trimester. Conversely, obesity was most often diagnosed during this trimester. There was a significant decrease in abnormalities of the skin and tongue at the post-partum examination. Gingival lesions — redness, swelling of the gums and pyorrhea — showed an increase of borderline significance during the third trimester and post partum. The incidence of edema and varicosities, as would be expected, was greater in the latter part of pregnancy, reaching a peak in the third trimester. A distinct decline occurred by the time of the post-partum examination. It would seem that increasing weight, particularly during the third trimester, more frequent skin disorders during pregnancy, and the increasing

incidence of edema and varicosities during the third trimester, with their return to more usual levels post partum, are all features that reflect the common physiological changes of pregnancy. The only change of possible nutritional significance was that occurring in the gingival lesions. These will be considered in detail in a later paper.

Age-parity relationships influenced the physical signs in all conditions except neurological abnormalities and night blindness. Table 13 illustrates the age and parity effects on the disorders recorded in table 12. In some it is evident that both age and parity effects are being exerted. There were no clear-cut parity effects entirely separable from age among the disorders examined. Age was responsible for the increased incidence of skin disorders, abnormalities of the eye (conjunctival injection and pingueculae), mouth disorders, adentia and edema. On the other hand, age and parity were interrelated in the findings concerning obesity, gum disorders, lesions of the tongue and varicose veins. As parity increased and the high parity group was subdivided more strictly, the above findings were even more accentuated.

In summary, the physical findings have been studied in relation to examiner differences, season, trimester, age and parity. It is evident that there are but few individual diagnoses that may be related to the nutritional status of pregnancy. The majority of significant differences were due either to individual examiner variations, to known physiologic processes occurring during the reproductive interlude, or to the closely interrelated factors of age and parity. No instances of clinically recognizable deficiency disease were encountered.

SUMMARY

The nutritional characteristics revealed by records of dietary intake, laboratory assessments and physical examinations are described for an unselected group of 2,129 pregnant white women.

These findings are discussed in relation to the altered physiology of pregnancy.

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THE BASAL METABOLIC RATE AND THE EFFECTS OF THIOURACIL ADMINISTRATION AND OF THYROIDECTOMY ON CONTROL AND VITAMIN B_6-DEFICIENT RATS

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(Received for publication June 10, 1953)

It has been reported (Beare et al., '53) that vitamin B_6-deficient rats exhibit a lower carcass content of total crude fatty acids than do pair-fed control animals, even when corn oil is included in the basal diet at a level of 20% by weight. Since the absorption of protein (Carter and Phizackerley, '51; Beaton et al., '53) and of fat and carbohydrate (Carter and Phizackerley, '51) is unaltered by vitamin B_6 deficiency, there are two possible explanations of the lower total crude fatty acid content of the body: (a) an increase in the basal metabolism of deficient rats, or (b) inefficient energy production in such animals. It has been reported (Orsini et al., '42) that vitamin B_6-deficient rats have a lower basal metabolic rate than do ad libitum-fed controls, but the effect of inanition was not determined. In one of the studies to be reported, the basal metabolic rate of vitamin B_6-deficient rats was compared with those of pair-fed and ad libitum-fed control animals. Further studies relating to energy metabolism are described, in which the effects of thiouracil administration and of thyroidectomy were investigated in control and vitamin B_6-deficient rats.

METHODS

Albino rats of the Wistar strain and of both sexes were housed in individual, screen-bottomed cages. One basal diet was employed throughout: the 20% corn oil, 20% casein, vitamin B_6-free diet previously described (Beaton et al., '53). All animals were provided with 100 μg of desoxypyridoxine per rat per day in the food to expedite the development of the deficiency syndrome. Control animals were further provided with 50 μg of pyridoxine hydrochloride per rat per day in the food. Drinking water was provided ad libitum.

For measurement of basal metabolic rate, an apparatus designed and constructed by the Department of Pharmacology of the University of Toronto, and kindly made available to us by Dr. J. K. W. Ferguson, was employed. This apparatus has been briefly described elsewhere (Ferguson and Sellers, '49). The equipment consisted of 4 lucite containers for individual rats, immersed in a temperature-controlled water bath set at 30° and connected to graduated glass cylinders in the water bath. Oxygen consumption was estimated by measuring the flow of water into the glass cylinders on the opening of the closed system to water, following a preliminary aeration period with a 95% oxygen: 5% carbon dioxide mixture. Measurements were made at 5-minute intervals and the reported data represent average oxygen consumptions per minute over a 20-minute period.

In the investigation of the effects of thiouracil administration and of thyroidectomy, analyses were carried out on blood, livers and carcasses. Blood was removed from the exposed heart, heparinized and pooled for each group. Livers were removed, pooled for each group, weighed and homogenized in a Waring Blendor. The remaining carcasses were pooled for each group, frozen in liquid air, passed through a power grinder and homogenized. All analyses were done in duplicate on aliquots of the pooled samples. The reported data represent averages of duplicate determinations.

On blood, the following analyses were performed by the procedures stated: amino nitrogen, Frame et al. ('43); urea,

Archibald ('45); plasma glutamic acid and glutamine, Prescott and Waelsch ('47); hemoglobin, Collier ('44); packed cell volume, by the standard procedure. Carcass nitrogen determinations were carried out with a standard macro-Kjeldahl procedure, multiplying the values found by the factor 6.25 to obtain protein content. A modified Liebermann procedure (Gavin and McHenry, '40) was employed for the

TABLE 1

Average basal metabolic rates[1] *of control and vitamin B_6-deficient rats*

DAYS ON DIET	GROUP	MALES	FEMALES	GROUP AVERAGE
0	Initial	149	156	152
5–7	Ad libitum-fed control	153	158	156
	Pair-fed control	146	142	144
	Deficient	144	145	145
12–14	Ad libitum-fed control	163	132	149
	Pair-fed control	130	132	131
	Deficient	128	131	130
19–21	Ad libitum-fed control	141	166	154
	Pair-fed control	126	136	131
	Deficient	134	130	132
26–28	Ad libitum-fed control	160	130	145
	Pair-fed control	102	103	103
	Deficient	100	98	99

[1] Expressed as oxygen consumption in milliliters per square meter of body surface per minute. Body surface was calculated from the formula $12.54W^{.66}$, where W represents body weight in grams.

estimation of total crude fatty acids in the carcass. Liver total vitamin B_6 was measured by a yeast microbiological method (Atkin et al., '43). Moisture content was determined by drying tissue in aluminum dishes for 5 hours in an electric oven at 105°.

EXPERIMENTAL

Vitamin B_6 deficiency and basal metabolic rate

Thirty-six rats were divided into three groups equal with respect to sex distribution, number and an initial average

body weight of 108 gm. The basal metabolic rate of one group was measured prior to placing the animals on experimental diets. All animals were then provided with the basal diet and with desoxypyridoxine. Two groups were further provided with pyridoxine, and served as controls. One control group and the deficient group were supplied with food ad libitum; the remaining control group's food intake was restricted to that of the deficient group to overcome differences due to inanition. Every week during an experimental feeding period of 4 weeks the basal metabolic rates of all groups were determined, employing 4 animals from each group on each of three consecutive days. During this experimental feeding period the average daily food intakes were 21 gm for the ad libitum-fed control group and 11 gm for the pair-fed control and deficient groups. The results of this investigation are shown in table 1. It should be noted that at the termination of the experiment all animals not provided with pyridoxine showed severe acrodynia.

Vitamin B_6 deficiency and thiouracil administration

Forty rats were divided into 4 groups equal with respect to sex distribution, number and an initial average body weight of 91 gm. All animals were provided with the basal diet and desoxypyridoxine; two groups were further provided with pyridoxine and were pair-fed with their comparable ad libitum-fed deficient groups. A solution of 0.25% thiouracil in water (as the sodium salt, pH 8) replaced the drinking water of one control and one deficient group. This dosage level and the method of administration of thiouracil were those employed by Williams et al. ('44). These workers observed cellular hyperplasia, decreased colloid and enlargement of the thyroid in young, thiouracil-treated Wistar strain rats within 16 days of treatment. No alterations were observed in any of the other numerous tissues examined, including endocrine glands (Williams et al., '44).

Following 19 days of experimental treatment, all animals were fasted for 18 hours and anesthetized by the intraperi-

TABLE 2

Average body weight changes, daily food consumptions and carcass and liver analyses

GROUP	FINAL NUMBER OF ANIMALS	AVERAGE BODY WEIGHT CHANGE	AVERAGE DAILY FOOD CONSUMPTION	CARCASS			LIVER TOTAL VITAMIN B_6	
				Total crude fatty acids	Protein	Moisture	Concentration	Content
		gm	*gm*	%	%	%	*μg/gm*	*μg*
Control	10	+ 38	7.7	6.8	22.1		8.5	42
Deficient	10	+ 9	7.7	3.8	23.9		5.4	23
Control + thiouracil	9	+ 23	6.6	8.4	21.8		8.1	47
Deficient + thiouracil	5	− 7	6.6	3.1	24.2		6.5	26
Control	10	+ 63	10.3	13.2	17.7	69.6	7.3	71
Deficient	9	+ 2	8.7	5.3	20.3	74.0	6.3	40
Control thyroidX [1]	9	− 13	5.5	6.6	18.9	73.2	9.0	37
Deficient thyroidX	9	− 26	3.9	4.6	20.0	73.7	9.0	30

[1] Thyroidectomized.

TABLE 3

Fasting blood metabolite levels

GROUP	BLOOD					PLASMA	
	Packed cell volume	Hemoglobin	Urea	Amino nitrogen	Sugar	Glutamic acid	Glutamine
	%	*gm %*	*mg %*	*mg %*	*mg %*	*mg %*	*mg %*
Control	46	13.1	28.6	10.6		5.5	
Deficient	43	12.1	36.6	11.7		4.2	
Control + thiouracil	40	11.9	49.0	8.4		1.2	
Deficient + thiouracil	27	7.7	78.6	0.9		1.0	
Control		12.1	29.8	15.0	112	5.6	14.7
Deficient		12.9	43.2	11.2	138	5.0	10.6
Control thyroidX [1]		11.5	40.2	9.3	104	4.9	8.6
Deficient thyroidX		11.5	51.4	10.0	120	6.0	14.9

[1] Thyroidectomized.

toneal administration of 2% butylone in saline. All animals not provided with pyridoxine had some degree of acrodynia, the acrodynia being most severe in those given thiouracil. During this experimental period 5 deaths occurred in the deficient group given thiouracil and one death in the control group given thiouracil. Examination suggested that the control animal's death was not due to dietary treatment. The results of analyses in this study are set down in tables 2 and 3.

Vitamin B_6 deficiency and thyroidectomy

Twenty-four rats of equal sex distribution were thyroidectomized by exposing and grasping each thyroid lobe with forceps. Between the removal of each lobe, the tips of the forceps were dipped in 0.9% saline to prevent the transfer of minute pieces of thyroid tissue which might act as grafts. Twenty-four rats of equal sex distribution were "sham-operated" by exposing but not removing the thyroid gland. Incisions were closed by stitching the muscle overlying the trachea and wound-clipping the skin. All animals were maintained on a stock chow and water regimen ad libitum for 6 to 7 days prior to the provision of the experimental diets. During this 6 to 7 days, and throughout the experimental feeding period, thyroidectomized animals were provided with 1% calcium lactate in water in place of drinking water, to reduce the incidence of death due to concomitant parathyroidectomy (Leblond and Eartly, '52).

Following the 6- to 7-day initial period, the animals were arranged in 4 groups of equal sex distribution and of average initial body weights as follows: sham-operated groups, 136 gm; thyroidectomized groups, 125 gm. All animals were provided with the basal diet and desoxypyridoxine. One sham-operated and one thyroidectomized group were further provided with pyridoxine. Since it was found that the food consumptions of the thyroidectomized groups were markedly irregular during the first 7 days of experimental feeding, all

groups were fed ad libitum rather than attempting a pair-feeding technique.

After 20 days of experimental feeding, all rats not provided with pyridoxine exhibited acrodynia, with no apparent difference in severity between the intact and thyroidectomized groups. During this 20-day period, three deficient and three control thyroidectomized rats died. One deficient intact rat died. The results of analyses in this study are set down in tables 2 and 3. Postmortem examination verified complete removal of thyroid tissue in all thyroidectomized animals and the parathyroid glands were found to be intact in almost all of these animals.

DISCUSSION

It is evident from the data in table 1 that transferring rats from a stock chow diet to the experimental diet fed on an ad libitum basis did not significantly alter their basal metabolic rate. Inanition appeared to cause a fall in basal metabolic rate within the first week of experimental feeding. It would seem that although the basal metabolic rate of vitamin B_6-deficient rats was lower than that of ad libitum-fed controls, this effect was a direct consequence of inanition. The report of others (Orsini et al., '42) that vitamin B_6 deficiency in the rat results in a lowered basal metabolic rate could be explained by inanition, since comparison was made with an ad libitum-fed control group. Under the experimental conditions employed here, no significant sex difference in basal metabolic rate was noted. The results of this study indicate that the failure of vitamin B_6-deficient rats to increase stores of body fat, as is evident in pair-fed controls (Beare et al., '53), is not due to an alteration in the basal metabolic rate.

Since the possibility existed that vitamin B_6-deficient rats have an impaired ability to produce energy efficiently, it was felt that an alteration in basal metabolism in such animals might alter the deficiency syndrome. This possibility was investigated by administering thiouracil in one experiment and by thyroidectomy in a second experiment. The biochemi-

cal lesions (Beaton et al., '53) and carcass composition (Beare et al., '53) of the vitamin B_6-deficient rat have been described in earlier reports and will not be discussed in any detail here. In the study presently reported, sub-normal body weight gains were observed in thiouracil-treated rats, as were noted by Williams et al. ('44). The severity of acrodynia and the incidence of mortality in the vitamin B_6-deficient, thiouracil-treated rats might be interpreted as an accentuation of the deficiency by thiouracil administration. Consideration must be given, also, to the possibility that thiouracil may have exerted a toxic effect, especially in the deficient group, distinct from any effect mediated through the thyroid gland. The marked hemodilution in the deficient group given thiouracil is difficult to explain simply on the basis of abnormal thyroid function. Beck et al. ('50) have studied the blood cell picture in control and vitamin B_6-deficient mice given thiouracil by injection and orally. These workers found that oral administration of thiouracil caused a decrease in food intake, which in turn caused a reduction in red and white cell counts and in hemoglobin concentration. No definitely beneficial effect of pyridoxine administration was noted. Since the results of the study reported here were suggestive, though not conclusive, a different procedure to obtain alteration of the basal metabolism was tested.

Thyroidectomy appeared to cause an elevation in fasting blood urea and a fall in fasting blood amino nitrogen concentrations in both deficient and control animals. These phenomena may be explained by the observation of Hoberman ('50) that, following thyroidectomy, amino acid catabolism is predominant over a smaller decrease in the rate of conversion of body protein to amino acids. A lowered hemoglobin concentration in both thyroidectomized groups was noted, but it cannot be stated that this represents anemia. It is of interest that although the average daily food consumption of the thyroidectomized deficient group was about half that of the intact deficient group, both groups had comparable carcass total crude fatty acid concentrations. Thy-

roidectomy elevated the liver concentrations, though not content, of total vitamin B_6 in both groups, and indeed the vitamin concentrations were identical in the two thyroidectomized groups. In thyroidectomized rats, vitamin B_6 deficiency caused a less marked increase in fasting blood urea than in intact rats. Leblond and Eartly ('52) have demonstrated the difficulty of completely depleting a rat of its stores of thyroid hormone. It is possible that, under these experimental conditions, the thyroidectomized animals still have some thyroid hormone stores.

SUMMARY

The basal metabolic rate of vitamin B_6-deficient rats was found not to be different from that of pair-fed controls, though lower than that of ad libitum-fed controls. Attempts to alter the basal metabolic rate either by the administration of thiouracil or by thyroidectomy did not cause definite alterations in the syndrome of vitamin B_6 deficiency.

ACKNOWLEDGMENT

This study was made possible by a grant from the National Vitamin Foundation.

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THE TOXICITY OF TRI-O-CRESYL PHOSPHATE FOR RATS AS RELATED TO DIETARY CASEIN LEVEL, VITAMIN E AND VITAMIN A [1]

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(Received for publication August 6, 1953)

The administration of tri-o-cresyl phosphate (TCP) to rabbits and rats was shown by Bloch ('42) and Bloch and Hottinger ('43) to cause muscular dystrophy, creatinurea, decreased plasma choline esterase and other symptoms of an acute vitamin E deficiency. The toxic reaction was preventable by additional vitamin E.

Similarly, the toxicities for rats of carbon tetrachloride, pyridine and benzene were decreased in severity by vitamin E under certain conditions of diet and dosage (Hove, '48, '53). The solvents accentuated symptoms of a vitamin E deficiency in rats. In all cases, the toxicity could be alleviated in part by raising the casein level of the diet. Therefore it was of interest to determine whether variations in dietary protein levels also influence the toxicity of TCP.

A property common to all of these toxic compounds, and of possible significance with respect to their toxic mechanism, is a strong pro-oxidant action in unsaturated fat systems. It has been shown (Hove, '53) that pyridine catalyzes oxygen-uptake in fat emulsions, *in vitro*. Data in the present paper

[1] Published with the approval of the Director of the Alabama Agricultural Experiment Station. This work was aided by a grant from the National Vitamin Foundation, and was presented at the annual meeting of the Federation of American Societies for Experimental Biology, Chicago, April, 1953. Vitamins were contributed by Merck and Company and the Staley Manufacturing Company.

show that several of the toxic chemicals, including TCP, catalyze the destruction of carotene in a linolate-hydroperoxide system. In view of these results, the toxicity of such compounds under certain conditions may be related not only to vitamin E but to vitamin A and unsaturated fatty acids as well. Hence, the effect of TCP on the utilization of vitamin A and of carotene has been determined.

Meunier et al. ('49) found vitamin A to exert a detoxicating action against bromobenzene in the growing rat. A very low plasma vitamin A level is characteristic of a hyperkeratotic condition in cattle called X-disease, a malady attributed to polychloronaphthalene (Sikes et al., '52; Groggins, '53). This chemical is a pro-oxidant *in vitro* and evidence is presented in the present paper that shows a low plasma tocopherol, as well as a low vitamin A level, in animals suffering from X-disease.

EXPERIMENTAL

The basal diet used for TCP-toxicity studies with rats contained methanol-extracted casein at 10, 20 or 60%; salt mixture (Salmon, '47), 4%; lard,[2] 9%; cod liver oil, 1%; and sucrose to make up 100%. Pure vitamins were ground with a portion of the sucrose to give the following levels per gram of diet: thiamine, riboflavin and pyridoxine, 5 μg each; calcium pantothenate, 30 μg; niacin, 40 μg; choline chloride, 2 mg; *i*-inositol, 0.2 mg; and 2-methyl, 1,4-naphthoquinone, 2 μg.

Weanling albino male rats were placed on the basal diet with 10% casein until the average weight had increased from 47 gm to 68 gm (18 days). Six rats were used for each of the 18 groups in the experiment; these were fed the three protein levels, with and without 0.1% TCP, and each of these levels with 0, 1, and 10 mg dl-α-tocopherol acetate per day by dropper in an olive oil solution. Body weight records were kept for 12 weeks.

[2] This lard was free from commercially added preservatives, and by analysis was found to contain 5.89% linoleic acid, 0.76% linolenic acid and 0.29% arachidonic acid.

At the termination of the experiment the rats were killed and the tissues prepared for determination of lipid composition. The livers and brains were separated, minced, weighed and homogenized with three times their volume in water. Absolute ethanol was added in an amount equal to the volume of the homogenate. After mixing, 100 ml of low-boiling petroleum ether were added and the mixture shaken for exactly 10 minutes. All analyses were carried out on aliquots of the separated petroleum ether phase. In preparing the whole carcass, the tail, head, viscera and visceral and loose fat were removed. The carcass was minced with a meat cleaver, ground three times through a cast-aluminum power grinder, and a 20-gm portion of the mince homogenized with 4 times its volume of water, and then treated in the same manner as the liver and brain samples. The dry matter of the ground carcass mince at the time of homogenization averaged 38.5% for all rats without vitamin E, and 39.8% and 42.3% for the rats receiving 1 and 10 mg of tocopherol, respectively.

All operations were carried out at room temperature (25°C.). The solvent was removed from aliquots under vacuum. Total fat was determined gravimetrically. Iodine numbers (Wijs) were determined by the Association of Official Agricultural Chemists method, but with all quantities reduced to one-tenth. Unsaturated fatty acids were determined by the method outlined by the Committee on Spectroscopic Methods ('49). The values were calculated and expressed as arachidonic, linolenic and linoleic acids, although it is clearly recognized that these are not exact classifications for several reasons. No attempt was made to estimate the fatty acids of unsaturation higher than tetraenoic. O'Connell, Lipscomb and Daubert ('52), among others, have commented on the disturbing influence of pentenoic and hexenoic acids on calculations for the tetraenoic and trienoic acids. The new and somewhat more exact calculation constants proposed by Brice et al. ('52) were not used. Determinations of pure linoleic acid [3]

[3] Hormel Foundation.

gave, with great precision, values 92 to 93% of theoretical values. The data obviously fall short of absolute accuracy, but the comparative nature of the experiment, combined with the high precision of the determinations, give merit to the data. Oleic acid was calculated from the iodine numbers, by difference. This value is also obviously inaccurate because of the uncertainty regarding hexenoic and other highly unsaturated acids. Total reducing substances, expressed as α-tocopherol, were determined by the Emmerie-Engel reaction in ethanol at the one-minute interval. The liver values were corrected for vitamin A interference by the method outlined by Quaife et al. ('49).

In determining the effect of TCP on the utilization of vitamin A and carotene, weanling male rats were depleted on a vitamin A-deficient diet with 20% casein but with only 5% lard and without cod liver oil. At depletion (average weight of 96 gm) the diet was supplemented with either 4.6 mg vitamin A acetate or 3.7 mg pure β-carotene per kilogram. In addition, some diets contained 0.1% TCP, while others contained 1.5% DL-methionine to depress growth and food consumption to the same extent as was noted with TCP diets. All diets contained 0.01% dl- α-tocopherol. Body weights and food consumption records were maintained for two weeks. The livers were extracted as previously indicated and analyzed for vitamin A by the antimony trichloride reaction without saponification. No carotenoids were present.

RESULTS

TCP toxicity as related to dietary casein and tocopherol levels

The average weekly gains for the rats on the various dietary treatments are given in table 1. At the level used, growth inhibition by TCP was only partially prevented by vitamin E even at high levels. However, increased casein levels increased the growth until nearly normal values were obtained. The effect of tocopherol became progressively less as the casein level was increased

The values in table 1 are based on a 6-week growth period, except for the groups on the lowest (10%) dietary casein level; these are based on a two-week period. All of the 6 rats on the 10% casein diet with TCP but without vitamin E died during the third and 4th weeks on experiment. On the TCP diet with 20% casein, two of the 6 rats died in the 9th and 12th week. On the control diet with 10% casein but without TCP or vitamin E, three of the 6 rats died in the 6th, 9th and 11th weeks. No other deaths occurred. At autopsy, all animals that died showed the typical massive

TABLE 1

Growth inhibition of rats by tri-o-cresyl phosphate (TCP) as related to dietary casein and vitamin E levels

CASEIN LEVEL IN DIET	AVERAGE WEEKLY GAIN								
	Controls (no TCP)			0.1% TCP in diet					
	mg α-tocopherol/day			mg α-tocopherol/day					
	0	1	10	0		1		10	
%	gm	gm	gm	gm	% [1]	gm	% [1]	gm	% [1]
10	10.5	12.6	12.5	4.0	(38.1)	8.4	(66.6)	8.1	(64.8)
20	32.2	34.3	33.7	19.3	(60.0)	25.5	(74.3)	24.7	(73.4)
60	30.0	34.8	34.5	24.8	(82.6)	29.0	(83.4)	30.8	(89.2)

[1] Values in parentheses are the gains as per cent of the gains made by the corresponding controls.

lung-hemorrhage-liver-necrosis syndrome reported by Hove, Copeland and Salmon ('49). None of the animals killed at termination had grossly evident lesions.

In a short study on the acute toxicity of TCP, this material was injected intraperitoneally as single doses, at levels of 1.0, 0.5 and 0.25 gm/kg body weight, into adult 300-gm male rats on the 10% casein diet without vitamin E. Deaths occurred between the second and 5th day after the injections, with the following rates: 4/4, 2/4 and 0/4 for the three levels, respectively. At autopsy the subcutaneous tissue was erythematous and had a bright rosy hue. Damage to the liver was characterized by bright red spots, about 1 mm in diameter and

evenly distributed in all lobes, on a yellowish-white background. The lungs were severely hemorrhaged.

In another short experiment, TCP was compared with tri-p-cresyl phosphate and tri-o-cresyl thiophosphate with respect to inhibition of rat growth. The respective 5-week average weight gains were — 4.0, 32.3 and 17.5, while controls gained 25.5 gm. These results were obtained with 8 rats per group, averaging 210 gm, on the 10% casein diet without vitamin E. The compounds were added to the diet at 0.1%.

TCP toxicity as related to tissue lipid composition

The composition of the lipids of liver, brain and carcass are given in table 2 (A, B and C). Several points are to be noted: The conjugated diene concentrations, presumably representing *in vivo* oxidation of linolate and hence a measure of tissue fat peroxides, were highest in rats fed TCP without vitamin E, and were somewhat elevated in control rats without vitamin E. Supplements of vitamin E lowered these values in all cases. The conjugated diene concentrations also decreased as the dietary casein levels were increased.

In general, the tocopherol content of tissue fat was depressed by TCP feeding. This was most evident in carcass and least evident in brain lipids. No explanation is at hand for the contrary values in the livers of rats on the 10% casein diets. The tissue tocopherols of the rats receiving the high vitamin E level (10 mg daily) are not given in the table, but were determined to establish absorption; for control rats on the 10, 20 and 60% casein diets, the average tocopherol values, as milligrams per gram of fat, were: 0.15, 0.26 and 0.29 for carcass; 0.50, 0.63 and 0.61 for brain; and 2.17, 2.57 and 2.34 for liver. Tissue tocopherols tended to be highest in rats on the higher casein diets.

The arachidonic acid concentrations in fats from rats fed TCP without vitamin E were consistently depressed. This was pronounced in carcass, somewhat less in brain, and least in liver fats. In all cases, rats fed vitamin E along with TCP had normal arachidonic acid values. In control rats, supple-

TABLE 2

Composition of tissue lipids

TCP IN DIET	CASEIN IN DIET	TOCOPHEROL DAILY (oral)	TOTAL LIPID (wet basis)	LIPID COMPOSITION				
				Oleic	Linoleic	Arachidonic	Conj. Diene	Tocopherol
%	%	mg	%	%	%	%	%	mg/gm
(A. Liver)								
0	10	0	2.91	12.2	13.57	9.21	0.76	0.75
0	10	1	6.43	32.0	12.61	10.50	0.28	1.07
0	20	0	1.73	10.5	10.15	14.90	0.42	0.89
0	20	1	2.06	31.4	12.74	15.68	0.23	0.82
0	60	0	2.49	9.2	13.36	15.20	0.26	1.95
0	60	1	2.55	19.6	12.49	16.91	0.29	2.32
0.1	10	0	1.68	...	15.23	10.56	1.95	0.83
0.1	10	1	6.14	10.2	11.56	13.42	0.29	1.17
0.1	20	0	3.75	11.1	12.49	14.38	0.42	0.60
0.1	20	1	3.94	34.6	12.36	17.81	0.29	0.41
0.1	60	0	3.52	17.4	9.98	14.60	0.48	0.63
0.1	60	1	2.83	33.7	8.97	16.61	0.41	1.09
(B. Brain)								
0	10	0	6.52	...	4.90	7.21	0.09	0.01
		1	5.82	...	5.03	8.75	0.07	0.43
0	20	0	5.69	64.0	5.46	11.06	0.06	0.15
		1	5.31	69.1	5.65	11.05	0.05	0.36
0	60	0	4.64	73.0	6.42	7.76	0.04	0.23
		1	4.26	71.5	6.05	7.80	0.04	0.58
0.1	10	0	6.85	...	4.03	4.02	1.40	0.00
		1	6.35	...	4.16	5.45	0.74	0.45
0.1	20	0	6.55	70.0	4.29	3.06	0.15	0.08
		1	6.06	62.3	7.90	10.41	0.03	0.34
0.1	60	0	6.48	70.9	4.49	4.76	0.16	0.02
		1	6.06	66.8	5.20	8.13	0.04	0.47
(C. Carcass)								
0	10	0	13.73	61.0	6.65	1.17	0.22	0.026
		1	13.58	59.5	5.64	1.18	0.18	0.068
0	20	0	13.18	61.5	5.52	1.30	0.20	0.023
		1	15.20	52.6	5.81	1.47	0.18	0.073
0	60	0	10.64	56.3	8.02	1.90	0.19	0.040
		1	13.50	56.5	8.17	1.88	0.12	0.101
0.1	10	0	7.89	...	6.64	0.63	1.08	0.000
		1	13.21	...	7.76	1.02	0.24	0.036
0.1	20	0	7.52	58.4	6.59	0.70	0.68	0.007
		1	17.72	57.0	6.90	1.25	0.21	0.044
0.1	60	0	6.36	24.4	7.07	0.99	0.37	0.000
		1	13.78	55.2	6.58	1.62	0.18	0.030

ments of vitamin E did not influence the arachidonic acid levels. However, it will be noted that the level of dietary casein had a marked effect; the values for carcass fat clearly showed that the arachidonic acid concentration increased as the casein level increased. This relation was evident to a lesser degree in the other tissue values.

Linolenic acid was not found in any fats analyzed except those from some of the TCP-fed rats without vitamin E and

TABLE 3

TCP toxicity as related to utilization of vitamin A acetate and β-carotene as measured by liver storage

(Vitamin A-depleted rats fed repletion diets for two weeks. Average weight at depletion was 96 gm. Five rats/group. The diet contained 0.01% dl-α-tocopherol)

ADDITION TO DIET	WEIGHT GAIN (2-wk.)	FOOD EATEN (2-wk.)	LIVER (WET BASIS)			UTILIZATION RATIO (% of ingested vitamin A or carotene found in liver)
			Weight	Fat	Vitamin A	
	gm	*gm*	*gm*	*%*	*μg/gm*	
(A. Rats fed vitamin A acetate at 4.6 mg/kg diet)[1]						
None	76.0	169	7.30	2.98	18.5	19.9 ± 1.83
TCP, 0.1%	35.2	136	7.50	4.00	9.32	12.9 ± 1.92
Methionine, 1.5%	36.4	108	5.88	5.11	19.2	26.3 ± 1.48
(B. Rats fed β-carotene at 3.7 mg/kg diet)[1]						
None	71.5	171	7.02	4.05	2.16	2.23 ± 0.34
TCP, 0.1%	32.8	134	5.81	4.52	00.58	0.63 ± 0.11
Methionine, 1.5%	34.0	126	5.32	5.31	3.25	2.97 ± 0.32

[1] Equivalent to 4.0 mg of vitamin A alcohol.

on the lower casein diets. In the 10% casein diet group, for example, the average linolenic acid of liver fat was 4.36%, and of carcass fats 0.17%. These fats were distinctly yellow when extracted from the tissues.

The oleic acid concentration of liver lipids was depressed in the absence of vitamin E, at all casein levels and with or without TCP. Linoleic acid was not consistently influenced by vitamin E, protein level or TCP feeding.

Fatty livers developed in rats on the control diet with 10% casein, a phenomenon noted by others (cf. Sauberlich, '53).

However, when vitamin E supplements were omitted, the fatty livers did not develop. This was previously reported by Hove and Hardin ('51). TCP fed to rats on the 20% casein diets caused an increase in liver fat concentration, and resulted in a marked decrease in carcass fat content unless vitamin E was fed.

TABLE 4

Pro-oxidant effect, in-vitro, of TCP and other toxic compounds

(The rates of carotene destruction in a system containing 20 μg of β-carotene and 90 mg of linoleate-hydroperoxide with 2,180 m. e. peroxide/kg made up to 10 ml with ethanol, and held at 30°C.).

COMPOUNDS ADDED AT 100 MG LEVELS TO CAROTENE SYSTEM	TIME REQUIRED FOR DESTRUCTION OF 50% OF THE CAROTENE
	hours
None	51.5
Tri-o-cresyl phosphate	16.0
Carbon tetrachloride	9.3
Pyridine	21.6
Benzene	23.5
Pentachloronaphthalene, commercial	19.5
Iso-octane	52.0
Tri-o-cresyl thiophosphate	29.5
Tri-p-cresyl phosphate	81.0
Chloroform	40.8
DDT	81.5
N-Hexane	51.8

TCP toxicity as related to the utilization of vitamin A and β-carotene

It is evident from the data in table 3 that TCP at 0.1% in the diet depressed carotene utilization drastically (28% of the control value), and depressed vitamin A utilization to a lesser degree (65% of the control value). Weight-restricted controls had normal liver vitamin A storage values.

Pro-oxidant effect of TCP and other chemicals, in vitro

The pro-oxidant effect of various compounds was determined in a system containing 20 μg of β-carotene in 9.9 ml of

absolute ethanol and 0.1 ml of methyl linolate hydroperoxide (peroxide number of 2,180 m.e./kg). The rate of carotene destruction at 30°C. was followed by measuring the yellow color of the solutions in the Evelyn colorimeter, filter 440, at various time intervals. The rate-of-destruction curves were plotted, and the point of 50% carotene loss noted from these curves. The addition to this system of TCP at a 100-mg level increased the rate of carotene destruction (table 4). Pyridine, benzene, CCl_4 and pentachloronaphthalene were also excellent catalysts. The pro-oxidant characteristics of these compounds have been studied in several systems and these data will be presented in detail elsewhere.

Plasma tocopherol of cattle with X-disease

As shown in table 4, chlorinated naphthalene is a pro-oxidant. This compound is said to be the causative agent of X-disease in cattle (Sikes et al., '52), a condition characterized by very low plasma vitamin A. Since TCP, which is thought of as an anti-vitamin E compound, also interferes with the utilization of carotene (table 3), it is of interest to note that in chlorinated naphthalene poisoning there is a decrease not only in the vitamin A but in the vitamin E concentration in plasma as well.

In the late fall of 1951, several cattle brought to the clinic of the School of Veterinary Medicine were diagnosed as suffering from severe cases of X-disease. Blood samples were analyzed for vitamin A, carotenoids and tocopherol.[4] The results are presented in table 5. The plasma tocopherol levels were decreased below normal. One animal recovered after several weeks' supplementation with 1 gm daily of natural α-tocopherol,[5] and showed an increased plasma level of fat-soluble vitamins. Three other animals, so supplemented, died within three days. While considerably more data are needed

[4] The blood samples were obtained through the courtesy of Dr. L. O. Llewellyn, of the Large Animal Surgery and Medicine Department.

[5] Obtained through the courtesy of Dr. P. L. Harris of Distillation Products Ind., Rochester, N. Y.

to relate X-disease to vitamin E metabolism conclusively, the general thesis appears to be supported; i.e., that the lipid-soluble toxic chemicals act through a pro-oxidant effect on unsaturated lipids and fat-soluble vitamins.

While all of the toxic chemicals studied may show a common pattern in their mode of action, there are obviously different points of attack, since each compound causes a somewhat different pathological picture.

TABLE 5

Plasma vitamin A, carotenoids and tocopherol of cattle suffering from X-disease

DESCRIPTION OF ANIMAL AND CONDITION	PLASMA LEVELS OF:		
	Vitamin A	Carotenoids	Tocopherol
	μg/100 ml	*μg/100 ml*	*μg/100 ml*
1. Normal yearling Hereford steer:	31.5	552	890
2. Yearling Hereford steer, with X-disease diagnosis:	5.9	445	250
3. Same animal 5 weeks after therapy with 1 gm d-α-tocopherol daily; and cured of X-disease:	15.9	434	450
4. Jersey heifer, with X-disease, died 4 days after therapy with 1 gm daily of α-tocopherol:	7.5	140	150
5. Black Angus, 3-month-old calf with X-disease; died:	20.6	175	125
6. Angus 3-month-old calf with very severe hyperkeratosis; died 2 days after tocopherol therapy:	0	19	0

DISCUSSION

All data obtained are consistent with the hypothesis that TCP exerts its toxic biological action through pro-oxidant activity in unsaturated lipid systems. Its presence in the diet of rats resulted in (a) increased conjugated dienes (fatty peroxides), (b) decreased arachidonic acid concentration, especially in carcass fats, (c) decreased tocopherol levels in tissue lipids, and (d) decreased liver storage of vitamin A after carotene feeding.

Although dietary vitamin E protected against certain phases of the TCP toxicity, notably the effect on tissue peroxides and arachidonic acid, it did not give complete protection against the growth inhibition. A factor necessary for protection against the growth inhibition due to TCP was present in casein. This may be the Factor 3 of Schwarz ('52). It is of interest to note that increased casein levels resulted in increased arachidonic acid concentrations in tissue lipids. Since the TCP toxicity decreased this factor, the increased arachidonate in rats on high casein diets may be directly related to the protective action of these diets.

The toxicity of TCP, CCl_4, pyridine, benzene and pentachloronaphthalene has been shown to involve lipid-soluble factors in the body. All of these toxic compounds are pro-oxidants *in vitro*, at least in certain unsaturated lipid systems. These two facts may possess more than a fortuitous relation.

SUMMARY

1. Growth inhibition of rats fed diets containing 0.1% of tri-o-cresyl phosphate was only partially prevented by vitamin E even at high levels. However, high dietary casein levels allowed almost normal growth even in the absence of vitamin E.

2. Liver, brain and carcass lipids from rats fed tri-o-cresyl phosphate showed increased conjugated dienes, decreased apparent arachidonic acid and decreased tocopherol levels. Supplements of vitamin E resulted in nearly normal values for these factors. Increased dietary casein levels, even without vitamin E, largely prevented the lipid changes. In control rats, increased casein levels resulted in an increased arachidonic acid content of tissues.

3. Tri-o-cresyl phosphate decreased liver vitamin A deposition in rats fed β-carotene or, to a lesser degree, vitamin A acetate.

4. *In vitro* studies revealed a pro-oxidant effect of trio-o-cresyl phosphate, carbon tetrachloride, pyridine and pentachloronaphthalene in catalyzing the destruction of carotene

in the presence of fat-peroxide. Limited data have shown that X-disease in cattle (chloronaphthalene poisoning) is associated with a decrease of plasma tocopherol as well as plasma vitamin A. An hypothesis is advanced relating the toxic mechanism of the fat-soluble toxic chemicals to their generalized oxidative action in unsaturated lipid systems.

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THE COMPARATIVE NUTRITIVE VALUE OF CORN OF HIGH AND LOW PROTEIN CONTENT FOR GROWTH IN THE RAT AND CHICK [1]

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(Received for publication April 20, 1953)

Since the early work of Hopkins (1899), numerous reports have appeared on the improvement of corn. However, most of these studies have been concerned with such factors as increased yields, lodging, resistance to disease and insects, maturity and appearance. In a number of studies the chemical constitution of the corn kernel has been investigated, particularly as to protein content (Smith, '12; Hume et al., '14; East and Jones, '20; Hayes, '22; Showalter and Carr, '22; Frey, '49, '51; Miller et al., '50; Hansen et al., '46; Earley and DeTurk, '50; Schneider et al., '52), and more recently with respect to some of the vitamins (Aurand et al., '50; Rodriguez et al., '50). These studies have been concerned primarily with the influence of genetic constitution and environmental factors. The effect of soil fertilization has been studied mainly from the standpoint of yields of corn, although a few reports have been made on the influence of soil fertility on kernel composition (Hamilton et al., '51; Schneider et al., '52). Only limited information is available on the influence of soil fertility and variety on the nutritive value of corn for experimental animals. In a preliminary report from this laboratory (Sauberlich, Chang and Salmon, '51),

[1] Published with the approval of the Director, Agricultural Experiment Station, Alabama Polytechnic Institute. This work was supported in part by a grant from the Williams-Waterman Fund of the Research Corporation.

differences in the nutritive value of low-protein and high-protein corn were noted in rat studies. Similar results have been reported recently by Mitchell, Hamilton and Beadles ('52) with rats and are also indicated in the swine studies of Dobbins et al. ('50a, b). Recently, additional samples of grain corn obtained from a study of the influence of variety, fertilization and plant spacing on the yield of corn were made available for further investigations. The results of these studies are reported in the present paper.

EXPERIMENTAL

The corn samples fed ranged widely in protein content. The samples considered as "low-protein" were obtained from variety test plots receiving a suboptimum level of nitrogen fertilization; the "high-protein" samples were from plots receiving 3.5 times as much nitrogen fertilization. The samples studied were obtained during the years 1948–50. In many instances, direct comparisons were obtained in feeding experiments with low- and high-protein samples of corn of the same variety. The low-protein samples ranged in protein from 6.8 to 9.1%, while the high-protein samples ranged from 9.5 to 13.6%. The additional nitrogen fertilization increased the protein content of the corn kernel (as determined by Kjeldahl nitrogen $\times$ 6.25) from 25 to 50%.

For the growth experiments, weanling rats of the Sprague-Dawley (SD) strain were placed individually in wire-bottomed cages. Food and water were given ad libitum and the animals were weighed weekly. Four rats were used per treatment group and nearly all experiments were repeated one or more times. The animals were fed the basal diet or a modification of it, as indicated, for a period of 4 weeks. The basal diet had the following percentage composition: ground corn grain (low- or high-protein content) 95.5, salts [2] 4, L-cystine 0.3, and choline chloride 0.2. The following vitamins were added, in milligrams per kilogram of diet: α-tocopherol ace-

[2] Salmon, W. D., J. Nutrition, *33:* 155, 1947.

tate 56.5, carotene (90% beta; 10% alpha) 5, calciferol 0.125, riboflavin 6, thiamine 3, pyridoxine 3, calcium pantothenate 15, niacin 25, inositol 300, folacin 1, biotin 0.5, and vitamin B_{12} 0.03.

Modifications of the diet consisted of amino acid supplements, replacement of part of the corn with sucrose or with protein supplements, or omission of certain vitamins. In

TABLE 1

Essential amio acid content of certain diets fed rats

AMINO ACID	AMINO ACID CONTENT (% OF DIET)[1]			
	95.5% Low-protein corn diet [2]	95.5% High-protein corn diet [2]	67.7% High-protein corn diet [2]	Rose's allowances
Arginine	0.44	0.58	0.41	0.20
Cystine	0.13 (0.3L)	0.21 (0.3L)	0.15 (0.3L)	
Methionine	0.16 (0.1DL)	0.21 (0.1DL)	0.15 (0.1DL)	0.40
Histidine	0.15 (0.1L)	0.24	0.17 (0.1L)	0.40
Isoleucine	0.26 (0.6DL)	0.42	0.30 (0.6DL)	0.50
Leucine	0.68	1.37	0.97	0.80
Lysine	0.22 (0.5L)	0.26 (0.5L)	0.18 (0.5L)	1.00
Phenylalanine	0.30	0.53	0.37	0.70
Tyrosine	0.27	0.45	0.32	
Threonine	0.36 (0.2DL)	0.51	0.36 (0.2DL)	0.50
Tryptophan	0.086 (0.1DL)	0.108 (0.1DL)	0.076 (0.1DL)	0.20
Valine	0.54 (0.2DL)	0.77	0.55 (0.2DL)	0.70

[1] Values in parentheses indicate level and isomer of amino acid used when added as supplements to the diets.

[2] Based on low-protein corn containing 7.8% protein and high-protein corn containing 11.4% protein.

many instances the amino acid supplements were added at the expense of the diet mixture. In table 1 are presented the essential amino acid contents of the basic diets fed, showing the effect the use of low- or high-protein corn had on the amino acid content of the diets. The values were obtained by microbiological assay methods (Sauberlich, Chang and Salmon, '53).

For the chick growth experiments, day-old Leghorn chicks of the Auburn strain were used. The chicks were divided into

groups of 10, placed in commercial batteries and supplied with water and feed ad libitum. The diet had the following composition (in grams): ground corn grain (low- or high-protein content) 80, soybean meal (41% protein) 10, meat and bone meal (50% protein) 3, salts [3] 4, cod liver oil 1, corn oil 2 and sucrose 2. The following vitamins were added, in milligrams per kilogram of diet: choline chloride 2,000, inositol 1,000, thiamine 6, riboflavin 6, pyridoxine 6, calcium pantothenate 30, niacin 50, α-tocopherol 25, α-tocopherol acetate 25, 2-methyl-1, 4-naphthoquinone 5, biotin 0.5, folacin 2 and vitamin B_{12} 0.03.

RESULTS

Diets containing equal percentages of corn grain

The results presented in table 2 demonstrate that the high-protein corn was superior to the low-protein corn when both were included in diets on an equal percentage basis. For instance, when the 95.5% corn diet (basal) was supplemented with methionine, lysine and tryptophan, amino acids considered low in corn, little or no growth was obtained when the low-protein corn was used, whereas moderate growth was obtained with the high-protein corn (2 gm/wk. compared with 23.5 gm/wk.; table 2, group 2). The supplementation of the low-protein corn diet with urea to furnish nitrogen equal to that in the high-protein corn diet failed to enhance the growth of the animals. However, the addition of the amino acids isoleucine, histidine, threonine and valine to the low-protein corn diet permitted moderate growth (19.2 gm/wk.; table 2, group 7).

Upon further study, it was revealed that the high-protein diet required amino acid supplements of only lysine and tryptophan to permit a moderate to good growth rate of rats. In contrast, the low-protein corn required supplements not only of tryptophan and lysine but also of threonine, isoleucine and valine (table 2). Supplements of cystine or methio-

[3] Schaefer, A. E., W. D. Salmon and D. R. Strength, Proc. Soc. Exp. Biol. Med., *77*: 202, 1949.

nine were apparently not necessary and histidine had only a slight effect. Although growth was poor when lysine or tryptophan was omitted from the diet, the high-protein corn appeared to support slightly better growth than the low-protein corn. Supplementation of the diets containing either low- or high-protein corn with amino acids also markedly improved the protein efficiency.

TABLE 2

Effect of amino acid supplementation on body weight gain of rats fed diets containing high- or low-protein corn

(8 rats per group)

GROUP NO.	DIET	LOW-PROTEIN CORN [1]		HIGH-PROTEIN CORN [1]	
		Ave. wt. gains/rat 4 wks.	Protein efficiency [2]	Ave. wt. gains/rat 4 wks.	Protein efficiency [2]
		gm		*gm*	
1	Basal diet (95.5% corn + 0.3% L-cystine)	5	...	16	...
2	Basal + 0.1% DL-methionine, 0.5% L-lysine, 0.1% DL-tryptophan	8	0.69	94	2.24
3	Same as 2, tryptophan omitted	5	0.34	17	0.74
4	Same as 2, + 1.12% urea	8	0.62	..	...
5	Same as 2, cystine and methionine omitted	..	...	98	2.65
6	Same as 2, lysine omitted	..	...	24	0.96
7	Same as 2, + 0.1% L-histidine, 0.2% DL-threonine, 0.2% DL-valine, 0.6% DL-isoleucine	77	2.97	..	...
8	Same as 7, isoleucine omitted	8	0.76	..	...
9	Same as 7, cystine and methionine omitted	81	3.44	..	...
10	Same as 7, lysine omitted	18	1.18	..	...
11	Same as 7, threonine omitted	9	0.42	..	...
12	Same as 7, valine omitted	48	2.41	..	...
13	Same as 7, histidine omitted	71	2.92	..	...

[1] Low-protein corn contained 7.8% protein; high-protein corn contained 11.4% protein.

[2] Expressed as ratio: $\frac{\text{gm gain in body weight}}{\text{gm of protein consumed}}$.

A very high incidence of fatty livers occurred in rats fed low-protein corn diets. The significance of this observation will be considered in a report of a more detailed investigation.

TABLE 3

Comparative weight gains of rats fed diets containing corn at iso-protein levels

(4 rats per group)

GROUP NO.[1]	DIET	PROTEIN CONTENT OF CORN FED	AVE. WT. GAIN/RAT 4 WKS.	PROTEIN EFFICIENCY [2]
		%	gm	
1-A	95.5% Corn + 0.5% L-lysine, 0.1% DL-tryptophan, 0.1% DL-methionine, 0.3% L-cystine	11.0	78	2.19
2-A	67.6% Corn + amino acids as in No. 1-A	11.0	2	0.19
3-A	95.5% Corn + amino acids as in No. 1-A	7.8	9	0.77
4-A	Same as No. 1-A + 0.1% L-histidine, 0.2% DL-threonine, 0.2% DL-valine, 0.6% DL-isoleucine	7.8	71	2.63
5-A	67.7% Corn + amino acids as in No. 4-A	11.0	62	1.92
6-B	95.5% Corn + 0.5% L-lysine, 0.1% DL-tryptophan	13.6	126	...
7-B	Same as No. 6-B	6.8	18	...
8-B	Same as No. 6-B + 0.2% DL-threonine, 0.2% DL-valine, 0.6% DL-isoleucine	6.8	62	...
9-B	47.75% Corn + amino acids as in No. 8-B	13.6	44	...

[1] Letters A and B refer to separate experiments.

[2] Expressed as ratio: $\frac{\text{gm gain in body weight}}{\text{gm of protein consumed}}$.

Diets containing equal percentages of corn grain protein

In further studies, high- and low-protein corn was fed on an equal protein basis in the diets. Under these conditions the low-protein corn proved to be superior to the high-protein corn (table 3). For example, when the low-protein corn was fed at an iso-protein level with the high-protein corn and

supplemented with the amino acids tryptophan, lysine, threonine, isoleucine and valine, the low-protein corn permitted considerably better growth in 4 weeks (table 3, groups 4-A vs. 5-A and 8-B vs. 9-B). Similar results are indicated in groups 2-A vs. 3-A when only tryptophan and lysine supplements were added. However, when the corn grain was fed on an equal weight basis, again note the marked superiority of the high-protein corn (table 3, groups 1-A and 3-A; 6-B and 7-B). Amino acid supplementation also improved the efficiency of the proteins in corn.

Supplementation of corn diets with protein and vitamin B_{12}

In additional experiments the corn diets were supplemented with natural protein materials instead of with purified amino acids. One of the objectives of these experiments was to determine whether or not the extra protein present in high-protein corn was of any value in lowering the amount of natural protein supplements normally required with corn diets. In table 4 (groups 1–8), the protein supplements were added to the corn diets at such a level that each supplement contributed the same amount of protein to the diet (either 2.5 or 5%). In addition, a comparison was made of the effectiveness of the protein supplements when added to diets containing either low-protein corn (7.1%) or high-protein corn (11.4%). All amino acid supplements, including cystine, were omitted from these diets. Soybean meal was found to be superior to the other protein supplements regardless of whether low- or high-protein corn was used. For instance, the low-protein corn-soybean meal diet (table 4, group 4) contained a total of 10.96% protein. The growth obtained on this diet (24.0 gm/wk.) is equal to that obtained in this laboratory with a 10% casein-sucrose diet supplemented with 0.3% L-cystine. This apparently reflects the ample amounts of lysine, tryptophan, threonine and isoleucine present in the soybean meal proteins. The poor growth obtained with the low-protein corn supplemented with meat and bone meal or blood powder was

probably due to an insufficient amount of isoleucine in the protein supplement. Similarly, the results with peanut meal supplementation (table 4, group 5) would indicate that the high-protein corn contained a greater amount of lysine than the low-protein corn, since peanut meal is deficient in this amino acid.

In each instance, better growth was obtained when the protein supplements were fed in the presence of the high-protein

TABLE 4

Comparative effect of protein supplements on the weight gains of rats fed diets containing low- or high-protein corn

(4 rats per group)

GROUP NO.	DIET [1]	AVE. WEIGHT GAIN/RAT/4 WKS.	
		Low-protein corn [2]	High-protein corn [2]
		gm	*gm*
1	84% Corn + 10% meat and bone meal	48	80
2	84% Corn + 12% cottonseed meal	64	76
3	84% Corn + 8.4% fish meal	64	72
4	84% Corn + 12% soybean meal	96	108
5	84% Corn + 10% peanut meal	48	88
6	90% Corn + 5% meat and bone meal	20	28
7	90% Corn + 5% blood powder	8	68
8	90% Corn + 4.2% fish meal	36	52

[1] The protein supplements were added to the diets at the expense of sucrose. The supplements added in groups 1–5 were calculated so that each contributed 5% of protein to the diets. In groups 6–8, the supplements contributed only 2.5% protein to the diets.

[2] The low-protein corn contained 7.1% protein; the high-protein corn contained 11.4% protein.

corn than when fed with the low-protein corn. Similarly, the low-protein corn required a greater amount of protein supplementation of the diet in order to obtain growth comparable to that obtained with the high-protein corn. These results would indicate a greater value for high-protein corn than for low-protein corn when used in the diet on a weight of grain basis.

The presence of vitamin B_{12} in the corn diets enhanced the growth of the rats by about 10%. Similar effects of vitamin

B_{12} were obtained with corn-soybean meal diets. Although the effect was not great, it was consistent (data not presented).

Growth of chicks fed diets containing low- or high-protein corn

The effect of the protein content of the corn was also demonstrated in feeding experiments with chicks (table 5). During a 5-week growth period, beginning with day-old chicks, the high-protein corn (12.0%) permitted an increase in body weight of about one-third over that obtained with the low-protein corn (7.7%). The results also reflect a greater value for high-protein corn over that of corn with a low protein content.

TABLE 5

Weight gains of day-old chicks fed diets containing low- or high-protein corn
(10 chicks per group)

DIET	PROTEIN CONTENT OF CORN FED	AVERAGE BODY WEIGHT AT			AVE. WT. GAIN/CHICK/ 5 WKS.[1]
		0 wk.	2 wk.	4 wk.	
	%	*gm*	*gm*	*gm*	*gm*
80% Low-protein corn diet	7.7	31	68	121	130 ± 12
80% High-protein corn diet	12.0	30	73	157	181 ± 14

[1] Average weight gains ± standard error of the mean; $P = < 0.02$.

DISCUSSION

From the results presented it appears that the increases of nitrogen in corn due to increased nitrogen fertilization represent true increases of proteins and amino acids in the corn and not merely that of non-amino acid nitrogen. In turn, the increased protein content of the corn influences its nutritive value. Although the high-protein corn was superior to the low-protein corn when fed on the basis of equal amounts of corn in the diet, the quality of the protein was decreased. Apparently nitrogen fertilization causes a greater increase in the amount of zein than in the amounts of the other proteins present. This appears to be substantiated by amino acid analyses (Sauberlich, Chang and Salmon, '53) and by the recent

report of Hamilton et al. ('51) that with proper soil and crop management the protein content of corn was increased, with the increase largely as zein. The study of Schneider et al. ('52) also indicated a similar effect of fertilization on the zein content of corn. Mitchell et al. ('52) have also noted that the biological value of corn decreases considerably as the protein content of corn increases, due apparently to an increased proportion of zein in the total proteins of corn. Thus the amount of lysine and tryptophan present per gram of protein becomes less, due to the absence (or nearly so) of these amino acids from zein. This explanation may also apply to the results of Dobbins et al. ('50a, b), who observed that low-protein corn was superior to high-protein corn when fed to swine on an equal protein basis. The genetic findings of various investigators (Showalter and Carr, '22; Frey, '49, '51; Hansen et al., '46; Schneider et al., '52) have also demonstrated that high-protein strains of corn contain a greater proportionate amount of zein than low-protein strains. Differences in the digestibility of the protein in low- and high-protein corn may possibly account partially for the effects noted in the present feeding experiments. However, Mitchell et al. ('52) noted that the digestibility of the mixed proteins of corn increased only slightly as the protein content of corn increased.

Although the effect of fertilization was primarily on the amount of zein present, it apparently increased somewhat the amounts of the other proteins present in corn. The feeding experiments indicated that the high-protein corn samples contained somewhat greater (although still deficient) amounts of lysine and tryptophan than the low-protein samples. Another favorable effect of nitrogen fertilization was the marked increase in the next limiting amino acids in corn; namely, isoleucine, threonine and valine.

Thus, increased rate of nitrogen fertilization not only increased the amount of protein present in corn but also increased the amounts of certain critical amino acids. From a practical feeding standpoint, these effects are important. Corn

is used for the most part as a feed on the weight-of-corn-grain basis. It furnishes not only a great part of the carbohydrates consumed but also an appreciable amount of protein. Under normal non-ruminant feeding conditions, protein supplements are required in corn diets to meet the protein and amino acid requirements of the animal. Therefore, any increases or changes in the protein content of corn may well alter the kind or reduce the amount and cost of the protein supplement required. Such effects were noted in the results obtained in the rat and chick experiments presented. However, the recent results of Barrentine et al. ('51) indicate that the additional protein in high-protein corn is of little value for the growth of chicks. This discrepancy in findings may be a result of the level and type of protein supplement used in the study of Barrentine et al. No details were given as to the composition of the commercial "chick concentrate" they used.

In ruminant feeding, where the amino acid and protein requirements are not so exacting, any increases in the protein content of corn may also reduce the amount of protein or nitrogen supplementation normally employed. Ross et al. ('52) recently reported that lambs fed either high- (13.2%) or medium-high-protein (11.03%) corn made significantly greater gains and produced significantly more wool than lambs fed low-protein (7.72%) corn. Earley and DeTurk ('50) have estimated that "if the protein content of the feed corn of the state of Illinois declined only one-half percent, which it probably has done, it would cost the feed industry $8,710,000 annually to replace it with soybean protein at 10 cents a pound."

SUMMARY

1. A series of corn samples, ranging in protein content from 6.8% to 13.6%, was employed in feeding experiments with rats and chicks. The low-protein varieties (range of 6.8 to 9.1% protein) were markedly increased in their protein content (9.5 to 13.6% protein) by increased rates of nitrogen fertilization.

2. The high-protein samples were superior to the low-protein samples when fed on the basis of equal proportions of corn grain in the diet. The low-protein corn was found to be deficient in at least the following amino acids: lysine, tryptophan, isoleucine, threonine and valine. In contrast, the high-protein corn was deficient in only lysine and tryptophan, although not as severely deficient as the low-protein corn.

3. However, when the corn was fed on the basis of equal protein content in the diet, the high-protein corn was somewhat inferior to the low-protein corn, reflecting a greater proportion of zein in the protein.

4. Studies on the supplementation of corn diets with various natural protein materials indicated that soybean meal is the most satisfactory of those tested. The protein supplements were most effective when added to the high-protein corn diets.

ACKNOWLEDGMENTS

The authors are indebted to Pryce B. Gibson, Department of Agronomy and Soils, of this Station, for assistance in obtaining the samples used in this study. The authors also express their appreciation to the Dow Chemical Company for generous supplies of methionine and tryptophan, to the Lederle Laboratories Division, American Cyanamid Company, for a supply of folacin, and to Merck and Company for all other vitamins used.

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